

INTERACTION OF BIOLOGICAL pH BUFFERS, USED IN BIOLOGICAL AND ENVIRONMENTAL STUDIES, WITH METAL IONS

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To my parents

The more you know who you are and what you want,
the less things upset you.

Bill Murray, in *Lost in Translation* from Sofia Coppola

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RESUMO

O catião hidrogénio tem um papel importante na química de soluções porque compete com os catiões metálicos presentes em solução. Assim, estudos envolvendo iões metálicos em solução, os quais são efectuados a pH constante, estão inevitavelmente sujeitos à possibilidade de interferências como resultado da complexação. Os tampões de pH estudados nesta tese cobrem uma vasta gama de pH usada em sistemas ambientais e biológicos.

Este trabalho teve como objectivo o estudo da interacção de três tampões de pH, ácido 2-hidroxi-3-[tris(hidroximetil)metilamino]-1-propanosulfónico (TAPSO), ácido [(2-hidroxi-1,1-bis(hidroximetil)etil)amino]-1-propanosulfónico (TAPS) e o ácido N-(1,1-dimetil-2-hidroxietil)-3-amino-2-hidroxipropanosulfónico (AMPSO) com seis catiões metálicos (Cd^{2+} , Co^{2+} , Cu^{2+} , Pb^{2+} , Ni^{2+} e Zn^{2+}), em solução aquosa, e a viabilidade da aplicação a estudos de especiação e estudos biológicos / bioquímicos envolvendo estes iões metálicos. Para tal, as constantes de estabilidade globais dos pares catião metálico / tampão de pH foram determinadas por duas técnicas independentes, a força iónica (nitrato de potássio 0.1 M) e temperatura (25.0 °C) constantes. A utilização de razões de concentração total de ligando / concentração total de metal, de várias ordens de grandeza, permitiu a caracterização correcta dos modelos finais. Foi ainda demonstrada a importância da análise gráfica dos dados e do uso de duas técnicas analíticas independentes.

Para os dezoito sistemas metal-tampão estudados, foi detectada complexação com diferentes graus de afinidade, tendo sido possível caracterizar cabalmente os modelos finais de treze sistemas. Não foi possível efectuar a caracterização completa de cinco sistemas (entre Co^{2+} ou Ni^{2+} e TAPS e Co^{2+} ou Ni^{2+} ou Pb^{2+} e AMPSO) devido à precipitação dos hidróxidos metálicos, $\text{M}(\text{OH})_2$. Os modelos finais e os valores das constantes de estabilidade globais variaram de acordo com a estrutura do tampão e com o ião metálico. A ordem de estabilidade dos complexos metal-tampão foi a seguinte: $\text{Cu}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$. Para todos os sistemas $\text{Cu}-(\text{tampão})_x-(\text{OH})_y$, os complexos CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ e $\text{CuL}_2(\text{OH})_2$ foram identificados. Para ambos os sistemas $\text{Pb}-(\text{tampão})_x-(\text{OH})_y$ caracterizados, os complexos PbL , $\text{PbL}_2(\text{OH})$ e $\text{PbL}_2(\text{OH})_2$ foram detectados, enquanto que para os sistemas $\text{Zn}-(\text{tampão})_x-(\text{OH})_y$ e $\text{Cd}-(\text{tampão})_x-(\text{OH})_y$, os complexos ZnL e $\text{ZnL}(\text{OH})_2$ e CdL foram identificados para todos os sistemas caracterizados. Outras espécies foram detectadas; o complexo ML_2 foi identificado nos sistemas $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ e $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$; a espécie CuL_3 formou-se no sistema $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$. As espécies $\text{ML}(\text{OH})_y$ foram identificadas para vários sistemas $\text{M}-(\text{tampão})_x-(\text{OH})_y$.

Simulações de equilíbrio químico, tendo em conta os valores mais frequentes de $\log K_{\text{ML}}$ descritos na literatura para ligandos naturais, comprovaram claramente que os três tampões podem interferir significativamente em estudos de especiação metálica. Este efeito parece ser mais notório para o sistema de $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$.

ABSTRACT

Hydrogen ion concentration plays an important role in the chemistry of aqueous solutions because it competes with metallic cations in solution for ligand binding sites. Thus, studies involving metal ions in solution, which require pH control, are inevitably subject to the possibility of buffer interferences as a result of complexation. The pH buffers studied in this thesis cover a large pH range, which embraces the range of pH that occurs in the environmental and biological systems.

The aim of this work was to study the interaction of three pH buffers, 2-Hydroxy-3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid (TAPSO), [(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]-1-propanesulfonic acid (TAPS) and N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid (AMPSO) with six divalent metals ions (Cd^{2+} , Co^{2+} , Cu^{2+} , Pb^{2+} , Ni^{2+} and Zn^{2+}), in aqueous solution, and their suitability for metal speciation and biological/biochemical studies involving the metal ions. The research was done in order to quantify the overall stability constants of metal ions and these pH buffers by two independent techniques, at a constant ionic strength (potassium nitrate 0.1 M) and 25.0 °C. The use of total ligand to total metal ion concentrations ratios of various orders of magnitude, and thus total metal ion concentration, allowed us to characterize properly the final models. The importance of graphic analysis of data and significance of employing two analytical techniques was demonstrated.

Complexation, with different strength, was detected for all eighteen $\text{M}-(\text{Buffer})_x-(\text{OH})_y$ systems. The final models of thirteen $\text{M}-(\text{buffer})_x-(\text{OH})_y$ systems, were fully defined whereas the complete characterization of five of these systems (between Co^{2+} or Ni^{2+} and TAPS and Co^{2+} or Ni^{2+} or Pb^{2+} and AMPSO) was not possible due to the early precipitation of metal hydroxide species. The final models and the values of the overall stability constants varied according to the structure of the buffer and the metal ion. The order of strength of the bound metal-buffer was: $\text{Cu}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$. For $\text{Cu}-(\text{buffer})_x-(\text{OH})_y$ systems, CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ were identified for all three systems studied. For both $\text{Pb}-(\text{buffer})_x-(\text{OH})_y$ systems, PbL , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$ were always detected, whereas for $\text{Zn}-(\text{buffer})_x-(\text{OH})_y$ and $\text{Cd}-(\text{Buffer})_x-(\text{OH})_y$ systems, only ZnL and $\text{ZnL}(\text{OH})_2$ and CdL were identified for all three $\text{M}-(\text{buffer})_x-(\text{OH})_y$ systems. The presence of other species was also detected; ML_2 was detected for $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ and $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ systems and CuL_3 was identified for $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ system. $\text{ML}(\text{OH})_x$ species were identified for several $\text{M}-(\text{buffer})_x-(\text{OH})_y$ systems.

Considering the frequency of $\log K_{\text{ML}}$ of natural ligands, reported in the literature, chemical simulations clearly evidenced that all three buffers can interfere to a significative extent in metal speciation studies. This effect seems to be more notorious for $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system.

RESUME

Le cation hydrogène joue un rôle important dans la chimie des solutions parce qu'il rivalise avec les cations métalliques présents en solution. Ainsi, les études qui englobent des ions métalliques en solution, lesquelles sont effectuées à pH constant, sont inévitablement sujettes à la possibilité d'interférences comme résultat de la complexation.

Les tampons de pH étudiés dans cette thèse couvrent une vaste gamme de pH utilisée dans des systèmes ambiants et biologiques.

Le but de ce travail a été l'étude de l'interaction de trois tampons de pH, l'acide 2-hydroxy-3-[tris(hydroxyméthyl)méthylamino]-1-propane sulfonique (TAPSO), l'acide [(2-hydroxy-1,1-bis(hydroxyméthyl)éthyl)amino]-1-propane sulfonique (TAPS) et l'acide N-(1,1-diméthyl-2-hydroxy-éthyl)-3-amino-2-hydroxy propane sulfonique (AMPSO) avec six cations métallique (Cd^{2+} , Co^{2+} , Cu^{2+} , Pb^{2+} , Ni^{2+} et Zn^{2+}), en solution aqueuse, et la viabilité de l'application à des études de spéciation et à des études biologiques / biochimiques qui englobent ces ions métalliques. Pour cela, les constantes de stabilité globales des paires cations métallique / tampon de pH, ont été déterminées par deux techniques indépendantes à force ionique (nitrate de potassium 0.1M) et température constantes. L'utilisation de rapports entre la concentration totale des ligands / concentration totale du métal avec plusieurs ordres de grandeur, a permis la caractérisation correcte des modèles finaux. Il a été aussi démontré l'importance de l'analyse graphique des données et de l'utilisation de deux techniques analytiques indépendantes. Dans les dix-huit systèmes métal / tampon étudiés, il a été détecté une complexation à différents degrés d'affinité, rendant possible la caractérisation parfaite des modèles finaux de treize systèmes. Il n'a pas été possible d'effectuer la caractérisation complète de cinq systèmes (entre Co^{2+} ou Ni^{2+} et TAPS et Co^{2+} ou Ni^{2+} ou Pb^{2+} et AMPSO) dû à la précipitation des hydroxydes métalliques, $\text{M}(\text{OH})_2$.

Les modèles finaux et les valeurs des constantes de stabilité globales ont varié selon la structure du tampon et selon l'ion métallique. L'ordre de stabilité des complexes métal / tampon a été la suivante: $\text{Cu}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$. Pour tous les systèmes $\text{Cu}-(\text{tampon})_x-(\text{OH})_y$, les complexes CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ et $\text{CuL}_2(\text{OH})_2$ ont été identifiés. Pour chacun des systèmes $\text{Pb}-(\text{tampon})_x-(\text{OH})_y$ caractérisés, les complexes PbL , $\text{PbL}_2(\text{OH})$ et $\text{PbL}_2(\text{OH})_2$ ont été détectés tandis que dans les systèmes $\text{Zn}-(\text{tampon})_x-(\text{OH})_y$ et $\text{Cd}-(\text{tampon})_x-(\text{OH})_y$, les complexes ZnL et $\text{ZnL}(\text{OH})_2$ et CdL ont été identifiés pour tous les systèmes caractérisés. D'autres espèces ont été détectées; le complexe ML_2 a été identifié dans les systèmes $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ et $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$; l'espèce CuL_3 s'est formée dans le système $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$. Les espèces $\text{ML}(\text{OH})_y$ ont été identifiées dans plusieurs systèmes $\text{M}-(\text{tampon})_x-(\text{OH})_y$.

Des simulations d'équilibre chimique, en tenant compte des valeurs plus fréquentes de $\log K_{\text{ML}}$ pour ligands naturels décrits dans la littérature, ont prouvé clairement que les trois tampons peuvent interférer significativement dans les études de spéciation métallique. Cet effet semble plus notoire pour le système de $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$.

PREFACE

The application of pH buffers to biological, biochemical, and environmental studies is a matter of routine. The quality of the results of those studies lies in the certainty that the pH buffers do not interact with the metals ions present in solution. However, this certainty proved to be not true to some extent. Therefore, the study of the interaction between commonly used pH buffers and several metals ions is fundamental to their proper use. This thesis may be considered a further development on the characterization of many (metal)–(buffers) systems, by determining the stability constants of the resultant complexes.

The present dissertation contains a series of papers that were written while the PhD has been carried out and are now published or submitted to publication. Some of the stability constants, published in meantime, were already compiled in The National Institute of Standards and Technology (NIST) Standard Reference Database.

This thesis has been developed, during the past four years, under the framework of the project POCTI/39950/QUI/2001, financed by “Fundação para a Ciência e a Tecnologia” and is divided into six parts. The first part contains a general introduction to the theme, with review on the literature and a brief description of the methodologies employed in the determination of the metal-buffer stability constants. Parts II to V are devoted to the characterization of the interaction of three pH buffers: TAPSO, AMPSO, and TAPS, with several important environmental and biological metals: copper (Part II), lead (Part III), cadmium and zinc (Part IV) and cobalt and nickel (Part V). Finally, in Part VI a general discussion and the main conclusions are presented outlining the most important aspects of the work. Also, some suggestions for future work are made.

CONTENTS

Acknowledgments.....	vii
Resumo	ix
Abstract.....	xi
Résumé	xiii
Preface.....	xv
Contents	xvii
List of Figures	xxiii
List of Tables	xxvii
Acrynomns	xxix
Symbols	xxxix
PART I Introduction.....	1
1 Introduction	3
1.1 Theoretical chemical support of a pH buffer.....	3
1.1.1 The importance of the pH maintenance.....	4
1.1.2 Good's buffers	5
1.2 Metal ion-buffer complexation studies.....	9
1.3 Methodology	16
1.4 Thesis aims.....	18
References	19
PART II Modelling Cu–(Buffer)_x–(OH)_y systems	25
2 Challenges in modelling and optimisation of stability constants in the study of metal complexes with monoprotonated ligands. a glass electrode potentiometric and polarographic study of a Cu–(TAPSO)_x–(OH)_y system.....	27
Abstract.....	27
2.1 Introduction.....	27
2.2 Experimental	28
2.2.1 Materials and Reagents.....	28
2.2.2 Apparatus.....	29
2.2.2.1 Polarography.....	29
2.2.2.2 Potentiometry	29
2.2.3 Procedure.....	29

2.2.3.1	Calibration of the Glass Electrode	29
2.2.3.2	Alternating current polarography.....	29
2.2.3.3	Direct current and differential pulse polarography	30
2.2.3.4	Glass Electrode Potentiometry.....	30
2.2.4	Data treatment	31
2.3	Results and Discussion.....	32
2.3.1	Protonation constants.....	32
2.3.2	Adsorption studies	33
2.3.3	Metal stability constants	34
3	Challenges in modelling and optimisation of stability constants in the study of metal complexes with diprotonated ligands. A glass electrode potentiometric and polarographic study of Cu-(AMPSO)_x-(OH)_y system.....	47
	Abstract.....	47
3.1	Introduction.....	47
3.2	Experimental	48
3.2.1	Materials and Reagents.....	48
3.2.2	Apparatus.....	48
3.2.2.1	Potentiometry	48
3.2.2.2	Polarography.....	49
3.2.3	Procedure.....	49
3.2.3.1	Calibration of the Glass Electrode	49
3.2.3.2	Alternating current polarography.....	49
3.2.3.3	Glass Electrode Potentiometry.....	49
3.2.3.4	Direct current and differential pulse polarography	50
3.2.4	Data treatment	50
3.3	Results and Discussion.....	51
3.3.1	Ligand adsorption and copper reversibility studies	51
3.3.2	Modelling procedures and refinement of stability constants	52
4	Challenges in modelling and optimisation of stability constants in the study of Cu-(TAPS)_x-(OH)_y system by polarographic.....	67
	Abstract.....	67
4.1	Introduction.....	67
4.2	Experimental	68
4.2.1	Materials and Reagents.....	68
4.2.2	Apparatus.....	69
4.2.2.1	Polarography.....	69
4.2.2.2	Potentiometry	69
4.2.3	Procedure.....	69
4.2.3.1	Calibration of the glass electrode.....	69
4.2.3.2	Alternating current polarography.....	69
4.2.3.3	Glass electrode potentiometry	70

4.2.3.4	Direct current polarography.....	70
4.2.4	Data treatment	70
4.3	Results and Discussion.....	71
4.3.1	Calculation of TAPS protonation constants	71
4.3.2	Ligand adsorption and reversibility studies	71
4.3.3	Prediction of species formed from DCP titrations.....	73
4.3.4	Modelling procedures and refinement of stability constants	75
4.3.4.1	DCP results.....	75
4.3.4.2	GEP results	77
4.3.5	Final model and overall stability constants values for Cu-(TAPS) _x -(OH) _y system.....	78
4.3.6	Comparative analysis of several Cu-(L) _x -(OH) _y systems with structural proposal	82
	References	87
	 PART III Modelling Pb-(Buffer)_x-(OH)_y systems	93
	 5 Graphic data analysis and complex formation curves as modelling and optimisation tools in potentiometric and voltammetric speciation studies of a Pb-(TAPSO)_x-(OH)_y system	95
	Abstract.....	95
5.1	Introduction.....	95
5.2	Experimental	96
5.2.1	Materials and reagents.....	96
5.2.2	Apparatus.....	97
5.2.2.1	Polarography.....	97
5.2.2.2	Potentiometry	97
5.2.3	Procedure.....	97
5.2.3.1	Calibration of the glass electrode.....	97
5.2.3.2	Alternating current polarography.....	97
5.2.3.3	Direct current and differential pulse polarography	97
5.2.3.4	Glass electrode potentiometry	98
5.2.4	Data treatment	98
5.3	Results and Discussion.....	99
5.3.1	Ligand adsorption and lead reversibility studies	99
5.3.2	Prediction of species formed from DCP titrations.....	101
5.3.3	Modelling procedures and refinement of stability constants	104
5.3.3.1	Polarography.....	104
5.3.3.2	Potentiometry	107
5.3.4	Overall stability constant values.....	109
	 6 Modelling of Pb-(TAPS)_x-(OH)_y system and refinement of stability constants, in the region of lead hydrolysis and lead hydroxide precipitation, by polarographic and potentiometric optimisation tools: importance of a multi-technique approach	113
	Abstract.....	113

6.1	Introduction	113
6.2	Experimental	114
6.2.1	Materials and Reagents.....	114
6.2.2	Apparatus.....	114
6.2.2.1	Polarography.....	115
6.2.2.2	Potentiometry	115
6.2.3	Procedure.....	115
6.2.3.1	Calibration of the glass electrode.....	115
6.2.3.2	Alternating current polarography.....	115
6.2.3.3	Direct current polarography.....	115
6.2.3.4	Glass electrode potentiometry	116
6.2.4	Data treatment	116
6.3	Results and Discussion.....	116
6.3.1	TAPS adsorption and lead reversibility studies.....	116
6.3.2	DCP graphical prediction of species present in solution	118
6.3.3	Modelling and optimization of the stability constants: DCP & GEP	120
6.3.4	Comparative evaluation of $Pb^{2+}-(L)_x-(OH)_y$ systems	125
	References	127
	 PART IV Modelling $Cd-(Buffer)_x-(OH)_y$ and $Zn-(Buffer)_x-(OH)_y$ systems.....	129
7	Interpretation of non-Nernstian slopes in graphic analysis of data collected in pH-range close to deprotonation of a ligand. Part I. A glass electrode potentiometric and polarographic study of $Cd-(TAPSO)_x-(OH)_y$ and $Zn-(TAPSO)_x-(OH)_y$ systems.....	131
	Abstract.....	131
7.1	Introduction.....	131
7.2	Experimental	132
7.2.1	Materials and reagents.....	132
7.2.2	Apparatus.....	132
7.2.2.1	Polarography.....	133
7.2.2.2	Potentiometry	133
7.2.3	Procedure.....	133
7.2.3.1	Calibration of the Glass Electrode	133
7.2.3.2	Alternating current polarography.....	133
7.2.3.3	Direct current and differential pulse polarography	133
7.2.3.4	Glass Electrode Potentiometry.....	134
7.2.4	Data treatment	134
7.3	Results and Discussion.....	135
7.3.1	Ligand adsorption and metals reversibility studies.....	135
7.3.2	Modelling of $Cd-(TAPSO)_x-(OH)_y$ system and refinement of stability constants	136
7.3.3	Modelling of $Zn-(TAPSO)_x-(OH)_y$ system and refinement of stability constants.....	145

8 Complex formation in the region of metal hydrolysis and $M(OH)_2$ precipitation. A glass electrode potentiometric and polarographic study of $Cd-(AMPSO)_x-(OH)_y$ and $Zn-(AMPSO)_x-(OH)_y$ systems 151

Abstract.....	151
8.1 Introduction.....	151
8.2 Experimental.....	152
8.2.1 Materials and reagents.....	152
8.2.2 Apparatus.....	152
8.2.2.1 Polarography.....	152
8.2.2.2 Potentiometry.....	153
8.2.3 Procedure.....	153
8.2.3.1 Calibration of the Glass Electrode.....	153
8.2.3.2 Alternating current polarography.....	153
8.2.3.3 Direct current polarography.....	153
8.2.3.4 Glass Electrode Potentiometry.....	154
8.2.4 Data treatment.....	154
8.3 Results and Discussion.....	155
8.3.1 Ligand adsorption and metals reversibility studies.....	155
8.3.2 Modelling and refinement of stability constants.....	156
8.3.2.1 $Cd-(AMPSO)_x-(OH)_y$ system.....	157
8.3.2.2 $Zn-(AMPSO)_x-(OH)_y$ system.....	163

9 Interpretation of non-Nernstian slopes in graphic analysis of data collected in pH-range of deprotonation of a ligand. Part II. A glass electrode potentiometric and polarographic study of $Cd-(TAPS)_x-(OH)_y$ and $Zn-(TAPS)_x-(OH)_y$ systems..... 169

Abstract.....	169
9.1 Introduction.....	169
9.2 Experimental.....	170
9.2.1 Materials and Reagents.....	170
9.2.2 Apparatus.....	170
9.2.2.1 Polarography.....	170
9.2.2.2 Potentiometry.....	171
9.2.3 Procedure.....	171
9.2.3.1 Calibration of the glass electrode.....	171
9.2.3.2 Alternating current polarography.....	171
9.2.3.3 Direct current polarography.....	171
9.2.3.4 Glass electrode potentiometry.....	172
9.2.4 Data treatment.....	172
9.3 Results and Discussion.....	172
9.3.1 Ligand adsorption and metal reversibility studies.....	172
9.3.2 Modelling and optimization of the stability constants for $M-(TAPS)_x-(OH)_y$ systems.....	173
9.3.2.1 $Cd-(TAPS)_x-(OH)_y$ system.....	175

9.3.2.2	Zn-(TAPS) _x -(OH) _y system	179
9.3.3	Correlation between the ligand basicity and complex stability of several CdL and ZnL systems	184
	References	187
 PART V Modelling Ni-(Buffer)_x-(OH)_y and Co-(Buffer)_x-(OH)_y systems.....		189
10	Complexation of cobalt and nickel ions by some zwitterionic biological buffers in aqueous solution: a potentiometric and spectrometric study.....	191
	Abstract.....	191
10.1	Introduction.....	191
10.2	Experimental.....	193
10.2.1	Materials and reagents.....	193
10.2.2	Apparatus.....	193
10.2.2.1	Potentiometry	193
10.2.2.2	UV-Vis spectroscopy	193
10.2.3	Procedure.....	193
10.2.3.1	Calibration of the glass electrode	193
10.2.3.2	Glass electrode potentiometry	193
10.2.3.3	UV-Vis spectroscopy	194
10.2.4	Data treatment	195
10.2.4.1	Glass Electrode Potentiometry	195
10.2.4.2	UV-Vis Spectroscopy.....	197
10.3	Results and Discussion	198
10.3.1	Modelling of M ²⁺ -(TAPSO) _x -(OH) _y systems and refinement of stability constants	198
10.3.1.1	M ²⁺ = Ni ²⁺	198
10.3.1.2	M ²⁺ = Co ²⁺	205
10.3.2	Modelling of M ²⁺ -(TAPS) _x -(OH) _y and M ²⁺ -(AMPSO) _x -(OH) _y systems.....	210
	References	213
 PART VI General discussion, Conclusions and Future work		215
11	General discussion, conclusions and future work.....	217
11.1	General discussion	217
11.2	Conclusions.....	226
11.3	Suggestions for future work.....	227
	References	229

LIST OF FIGURES

Figure 1-1 Useful pH ranges of some selected biological buffers, pKa values, and the acronyms by which Good buffers and some other useful biological buffers are known	7
Figure 2-1 Structure of 2-hydroxy-3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid (TAPSO).	32
Figure 2-2 AC polarograms for TAPSO solutions in 0.1 M KNO ₃ at pH 5.55.....	33
Figure 2-3 Observed shift in the DCP half-wave potential as a function of pH and the free ligand concentration for Cu-(TAPSO) _x -(OH) _y system.	36
Figure 2-4 Results from refinement and optimisation operations obtained from DCP and GEP experiments for Cu-(TAPSO) _x -(OH) _y system.....	39
Figure 2-5 Species distribution diagrams computed for Cu-(TAPSO) _x -(OH) _y system: [L _T]:[Cu _T] = 3, [Cu ²⁺] = 9.442 × 10 ⁻⁴ M, [L _T]:[Cu _T] = 4, [Cu ²⁺] = 9.442 × 10 ⁻⁴ M, and [L _T]:[Cu _T] = 100, [Cu ²⁺] = 5 × 10 ⁻⁵ M.	44
Figure 2-6 Species distribution diagrams computed, for the final model, for Cu-(TAPSO) _x -(OH) _y system. [L _T]:[Cu _T] = 2, [Cu ²⁺] = 9.442 × 10 ⁻⁴ M, [L _T]:[Cu _T] = 3, [Cu ²⁺] = 9.442 × 10 ⁻⁴ M, [L _T]:[Cu _T] = 4, [Cu ²⁺] = 9.442 × 10 ⁻⁴ M, and [L _T]:[Cu _T] = 100, [Cu ²⁺] = 5 × 10 ⁻⁵ M.	46
Figure 3-1 Illustration of the fitting procedure of the DC polarograms for Cu-(AMPSO) _x -(OH) _y system.....	52
Figure 3-2 Z _M function for Cu-(AMPSO) _x -(OH) _y system calculated from the refinement operations performed on the GEP data by ESTA program.....	53
Figure 3-3 Analysis of the DCP data obtained from the Cu-(AMPSO) _x -(OH) _y system at 25°C and 0.1 M KNO ₃ ionic strength.....	55
Figure 3-4 Species distribution diagrams computed for different Cu-(AMPSO) _x -(OH) _y models. A, C and E – GEP experimental conditions, [L _T]:[Cu _T] = 9.1, [Cu ²⁺] = 8.97 × 10 ⁻⁴ M; B, D and F – DCP experimental conditions, [L _T]:[Cu _T] = 155, [Cu ²⁺] = 4.9 × 10 ⁻⁵ M.....	63
Figure 4-1 AC polarograms for TAPS solutions in 0.1 M KNO ₃ at pH = 10.34.....	72
Figure 4-2 Prediction of species for Cu-(TAPS) _x -(OH) _y system, from DCP data	74
Figure 4-3 Species distribution diagram for Cu-(TAPS) _x -(OH) _y system. (A) [L _T]:[Cu _T] = 97.5, [Cu ²⁺] = 4.0 × 10 ⁻⁵ M; (B) [L _T]:[Cu _T] = 10, [Cu ²⁺] = 1.0 × 10 ⁻³ M.	79
Figure 4-4 Species distribution diagram for the final model of Cu-(TAPS) _x -(OH) _y system. (A) [L _T]:[Cu _T] = 10, [Cu ²⁺] = 1.0 × 10 ⁻³ M; (B) [L _T]:[Cu _T] = 97.5, [Cu ²⁺] = 4.0 × 10 ⁻⁵ M; (C) [L _T]:[Cu _T] = 151.6, [Cu ²⁺] = 7.0 × 10 ⁻⁶ M.	81
Figure 4-5 Relationship between log β _{CuL} and pKa for Cu ²⁺ and several biological buffers and other amines....	84
Figure 4-6 Proposed structures for Cu(TAPSO) ⁺ and Cu(TAPS) ⁺ complexes.....	85

Figure 5-1 Variation of the capacitive current recorded by ACP, without lead ion, with the increase of TAPSO concentration, at three pH values	100
Figure 5-2 Analysis of the DPP and DCP data for Pb-(TAPSO) _x -(OH) _y system.....	103
Figure 5-3 Experimental and calculated complex formation curves obtained for the models indicated in the figure for DCP and GEP data for Pb-(TAPSO) _x -(OH) _y system.....	105
Figure 5-4 Species distribution diagrams as a function of pH calculated for the Pb-(TAPSO) _x -(OH) _y system based on DCP and GEP results reported in this work at 25.0 °C and 0.1 M KNO ₃	110
Figure 5-5 Species distribution diagrams as a function of pH computed for Pb-(TAPSO) _x -(OH) _y system.....	112
Figure 6-1 Variation in the capacitive current recorded by ACP with the increase of TAPS concentration at three pH values, 6.34, 8.34 and 10.34.....	117
Figure 6-2 Interpretation of the DCP experimental data for Pb-(TAPS) _x -(OH) _y system involving the corresponding slopes and electrochemical reactions at the DME electrode surface for [L _T]:[Pb _T] = 88.7, [Pb ²⁺] = 9.6 × 10 ⁻⁶ M, 0.1 M KNO ₃ at 25.0°C.....	119
Figure 6-3 Experimental and calculated complex formation curves for Pb-(TAPS) _x -(OH) _y system obtained for the models indicated in the figure for DCP data: [L _T]:[Pb _T] = 88.7, [Pb ²⁺] = 9.6 × 10 ⁻⁶ M, at 25.0°C and 0.1 M KNO ₃	121
Figure 6-4 Species distribution diagrams computed for Pb-(TAPS) _x -(OH) _y system.....	124
Figure 7-1 Typical DC polarograms of zinc in the presence of TAPSO with the increase of pH.....	136
Figure 7-2 Graphic analysis of the data obtained for the Cd-(TAPSO) _x -(OH) _y system by DPP.....	138
Figure 7-3 Modelling of the Cd-(TAPSO) _x -(OH) _y system by use of complex formation curves.	140
Figure 7-4 Species distribution diagrams for Cd-(TAPSO) _x -(OH) _y system, computed for (A) [L _T]:[Cd _T] = 308, [Cd ²⁺] = 5.0 × 10 ⁻⁵ M and (B) [L _T]:[Cd _T] = 3.3, [Cd ²⁺] = 3.0 × 10 ⁻³ M.	144
Figure 7-5 Corrected half-wave potential as a function of pH and as a function of the free ligand concentration obtained by DCP for Zn-(TAPSO) _x -(OH) _y system	145
Figure 7-6 Experimental and calculated complex formation curves for the Zn-(TAPSO) _x -(OH) _y system.	147
Figure 7-7 Species distribution diagrams for the Zn-(TAPSO) _x -(OH) _y system.....	150
Figure 8-1 Variation of the capacitive current recorded by ACP, without metal ions, with the increase of AMPSO concentration, at pH 9.0	156
Figure 8-2 Half-wave potential shift as a function of pH and the free ligand concentration, recorded by DCP, for Cd-(AMPSO) _x -(OH) _y system, and Zn-(AMPSO) _x -(OH) _y system.	158
Figure 8-3 Experimental and calculated complex formation curves for DCP data: (A) Cd-(AMPSO) _x -(OH) _y system, and (B) Zn-(AMPSO) _x -(OH) _y system.....	160
Figure 8-4 Species distribution diagrams computed for Cd-(AMPSO) _x -(OH) _y system.....	162

Figure 8-5 Species distribution diagrams computed for Zn-(AMPSO) _x -(OH) _y system.	166
Figure 9-1 Variation in the capacitive current recorded by ACP, without the metal ion, with the increase of TAPS concentration at different pH values.	174
Figure 9-2 Analysis of DCP data obtained from the Cd-(TAPS) _x -(OH) _y system	176
Figure 9-3 Species distribution diagrams for the final model for Cd-(TAPS) _x -(OH) _y system.....	178
Figure 9-4 Analysis of DCP data obtained from the Zn-(TAPS) _x -(OH) _y system	180
Figure 9-5 Species distribution diagrams for the final model for Zn-(TAPS) _x -(OH) _y system.....	183
Figure 9-6 Relationship between log K_{ML} and p <i>Ka</i> for Cd ²⁺ (A) and Zn ²⁺ (B) 1:1 complexes of several biological buffers	186
Figure 10-1 Structures, p <i>Ka</i> values and pH range of the buffers tested in this work.	192
Figure 10-2 Results from refinement and optimization operations obtained from GEP data for Ni-(TAPSO) _x -(OH) _y , Co-(TAPSO) _x -(OH) _y , Ni-(TAPS) _x -(OH) _y , Ni-(AMPSO) _x -(OH) _y .systems	201
Figure 10-3 Spectrophotometric curves of the titrations: (A) [TAPSO _T]:[Ni _T] = 2.0, [Ni ²⁺] = 3.0 × 10 ⁻² M, pH between 3.0 and 7.6; (B) [TAPSO _T]:[Co _T] = 5.2, [Co ²⁺] = 1.5 × 10 ⁻² M, pH between 5.0 and 7.9.	202
Figure 10-4 Species distribution diagrams plotted for the final model for Ni-(TAPSO) _x -(OH) _y system.....	205
Figure 10-5 Species distribution diagrams plotted for the final model for Co-(TAPSO) _x -(OH) _y system.....	210
Figure 11-1 Fraction of each species in a system containing 0.01 M buffer at pH 8.0 in the presence of a equivalent molar concentration, 6.0 nM, of natural organic ligands (L1) and copper: (A) TAPSO; (B) TAPS; (C) AMPSO.	222
Figure 11-2 Fraction of each species in a system containing 0.01 M TAPSO at pH 8.0 in the presence of equivalent molar concentration of natural organic ligands (L1) and the following metal ions: (A) [L1] or [Pb ²⁺] = 0.6 nM; (B) [L1] or [Zn ²⁺] = 6.0 nM TAPS; (C) [L1] or [Cd ²⁺] = 0.6 nM.	224

LIST OF TABLES

Table 1-1 Common biological buffers and theirs pK_a values at 25.0°C and 0.1M ionic strength.....	5
Table 1-2 Structure, pK_a and stability constants values (as $\log \beta$) of morpholinic family buffers.....	10
Table 1-3 Structure, pK_a and stability constants values (as $\log \beta$) of piperazinic family buffers.	11
Table 1-4 Structure, pK_a and stability constants values, as $\log \beta$, of bis(2-hydroxyethyl)amine family buffers ...	12
Table 1-5 Structure, pK_a and stability constants values (as $\log \beta$) of TRIS family buffers	13
Table 1-6 Structure, pK_a and stability constants values (as $\log \beta$) of some biological buffers	14
Table 1-7 Structure, pK_a and stability constants values (as $\log \beta$) of Cyclohexylamino family buffers.....	15
Table 2-1 Dissociation constant for water, protonation constant for the ligand TAPSO and overall stability constants for Cu^{2+} complexes with OH^- , at 25 °C.....	32
Table 2-2 Overall stability constants for $Cu-(TAPSO)_x-(OH)_y$ system determined at 25 °C and $I = 0.1$ M by DCP for $[L_T]:[Cu_T] = 50$, $[Cu^{2+}] = 2.0 \times 10^{-5}$ M.....	38
Table 2-3 Overall stability constants for $Cu-(TAPSO)_x-(OH)_y$ system determined by GEP, at $[Cu^{2+}] = 9.44 \times 10^{-4}$ M, at 25 °C and $I = 0.1$ M.....	42
Table 3-1 Protonation constant for the ligand AMPSO, at 25 °C.....	51
Table 3-2 Overall stability constants for $Cu-(AMPSO)_x-(OH)_y$ system refined with ESTA program from GEP data.	58
Table 3-3 Overall stability constants for $Cu-(AMPSO)_x-(OH)_y$ system obtained from DCP data, 25 °C and 0.1 M KNO_3	60
Table 3-4 Overall stability constants for copper complexes with AMPSO obtained in this work, after combining polarographic and potentiometric results, at 25 °C and 0.1 M ionic strength.	64
Table 4-1 Protonation constant for the ligand TAPS.....	71
Table 4-2 Overall stability constants for $Cu-(TAPS)_x-(OH)_y$ system determined by DCP.....	76
Table 4-3 Comparison between CuL complex stability constants and basicity for some biological buffers and other amines.....	83
Table 5-1 Overall stability constants for Pb^{2+} complexes with OH^- , at 25 °C.....	99
Table 5-2 Overall stability constants for $Pb-(TAPSO)_x-(OH)_y$ system determined at 25 °C and $I = 0.1$ M by DCP.....	104
Table 5-3 Refinement strategy for GEP titrations. Overall stability constants for $Pb-(TAPSO)_x-(OH)_y$ system	108
Table 6-1 Refinement of the stability constants (as $\log \beta$) obtained for $Pb-(TAPS)_x-(OH)_y$ system by polarography and by potentiometry..	122

Table 6-2 Stability constants (as $\log \beta$) for $\text{Pb}^{2+}\text{-(L)}_x\text{-(OH)}_y$ systems.....	125
Table 7-1 Overall stability constants for Cd^{2+} and Zn^{2+} complexes with OH^- , at 25 °C..	135
Table 7-2 Overall stability constants (as $\log \beta$ values) for $\text{Cd-(TAPSO)}_x\text{-(OH)}_y$ system determined at 25 °C and $\mu = 0.1$ M by DPP and by GEP	141
Table 7-3 Overall stability constants (as $\log \beta$ values) for $\text{Zn-(TAPSO)}_x\text{-(OH)}_y$ system, determined at 25 °C and $\mu = 0.1$ M by DCP and by GEP.....	148
Table 8-1 Overall stability constants for $\text{Cd-(AMPSO)}_x\text{-(OH)}_y$ system determined at 25 °C and $I = 0.1$ M by DCP and GEP	149
Table 8-2 Stability constants obtained for $\text{Zn-(AMPSO)}_x\text{-(OH)}_y$ system, determined at 25 °C and $I = 0.1$ M by DCP and GEP	149
Table 9-1 Refinement of the stability constants (as $\log \beta$) for $\text{Cd-(TAPS)}_x\text{-(OH)}_y$ system by DCP and GEP..	149
Table 9-2 Refinement of the stability constants (as $\log \beta$) for $\text{Zn-(TAPS)}_x\text{-(OH)}_y$ system by DCP and GEP ..	149
Table 9-3 Comparison between CdL and ZnL complex stability constants and basicity for some biological buffers and other amines.....	149
Table 10-1 Overall stability constants for Ni^{2+} and Co^{2+} complexes with OH^- , at 25.0 °C [14].	149
Table 10-2 Overall stability constants (as $\log \beta$ values) for $\text{Ni-(TAPSO)}_x\text{-(OH)}_y$ system determined at 25.0 °C by GEP	199
Table 10-3 Overall stability constants (as $\log \beta$ values) for $\text{Ni-(TAPSO)}_x\text{-(OH)}_y$ system determined at 25.0 °C by UV-Vis spectroscopy.	149
Table 10-4 Overall stability constants (as $\log \beta$ values) for $\text{Co-(TAPSO)}_x\text{-(OH)}_y$ system determined at 25.0 °C by GEP	206
Table 10-5 Overall stability constants (as $\log \beta$ values) for $\text{Co-(TAPSO)}_x\text{-(OH)}_y$ system determined at 25.0 °C by UV-Vis spectroscopy	149
Table 11-1 Final models and stability constants values (as $\log \beta$) for all $\text{M-(L)}_x\text{-(OH)}_y$ systems studied.	149

ACRYNOMS

ACES	<i>N</i> -(2-Acetamido)-2-aminoethanesulfonic acid; <i>N</i> -(Carbamoylmethyl)-2-aminoethanesulfonic acid; <i>N</i> -(Carbamoylmethyl)taurine
ACP	Alternating current polarography
ADA	<i>N</i> -(2-Acetamido)iminodiacetic acid; <i>N</i> -(Carbamoylmethyl)iminodiacetic acid
AMP	2-Amino-2-methyl-1-propanediol
AMPD	2-Amino-2-methyl-1,3-propanediol
AMPSO	3-([1,1-Dimethyl-2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid <i>N</i> -(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid
BES	<i>N,N</i> -Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; <i>N,N</i> -Bis(2-hydroxyethyl)taurine
Bicine	<i>N,N</i> -Bis(2-hydroxyethyl)glycine
BIS – TRIS	2,2-Bis(hydroxymethyl)-2,2',2''-nitrilotriethanol; 2-Bis(2-hydroxyethyl)amino-2-(hydroxymethyl)-1,3-propanediol; Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane
CABS	4-(Cyclohexylamino)-1-butanesulfonic acid
CAPS	3-(Cyclohexylamino)-1-propanesulfonic acid
CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid
CCFC	Calculated complex formation curve
CHES	2-(Cyclohexylamino)ethanesulfonic acid
Citric acid	2-Hydroxypropane-1,2,3-tricarboxylic acid
DCP	Direct current polarography
DME	Dropping mercury electrode
DPP	Differential pulse polarography
DIPSO	3-(<i>N,N</i> -Bis[2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid; <i>N,N</i> -Bis(2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid
ECFC	Experimental complex formation curve
EPPS	4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid; 4-(2-Hydroxyethyl)piperazine-1-propanesulfonic acid; <i>N</i> -(2-Hydroxyethyl)piperazine- <i>N'</i> -(3-propanesulfonic acid); HEPPS
ESR	Electron spin resonance
GEP	Glass electrode potentiometry

Gly-Gly	Diglycine; Glycyl-glycine
HEPBS	N-(2-Hydroxyethyl)piperazine-N'-(4-buthanesulfonic acid)
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid; N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
HEPPSO	N-(2-Hydroxyethyl)piperazine-N'-(2-hydroxypropanesulfonic acid); 4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid)
MES	2-(N-Morpholino)ethanesulfonic acid; 4-Morpholineethanesulfonic acid
MOBS	4-(N-Morpholino)buthanesulfonic acid
MOPS	3-(N-Morpholino)propanesulfonic acid; 4-Morpholinepropanesulfonic acid
MOPSO	β -Hydroxy-4-morpholinepropanesulfonic acid; 3-Morpholino-2-hydroxypropanesulfonic acid
NMR	Nuclear magnetic resonance
PIPES	1,4-Piperazinediethanesulfonic acid; Piperazine-1,4-bis(2-ethanesulfonic acid); Piperazine-N,N'-bis(2-ethanesulfonic acid)
POPSO	Piperazine-1,4-bis(2-hydroxypropanesulfonic acid); Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid)
SDD	Species distribution diagram
TABS	N-tris(Hydroxymethyl)methyl-4-aminobuthanesulfonic acid
TAPS	[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]-1-propanesulfonic acid; N-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid
TAPSO	2-Hydroxy-3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid; N-[Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid
TES	2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid; N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid
Tricine	N-[Tris(hydroxymethyl)methyl]glycine
TEA	Triethanolamine; 2,2',2''-Nitrilotriethanol; Tris(2-hydroxyethyl)amine
Trizma	2-Amino-2-(hydroxymethyl)-1,3-propanediol; THAM; Tris base; Tris(hydroxymethyl)aminomethane; Trometamol

SYMBOLS

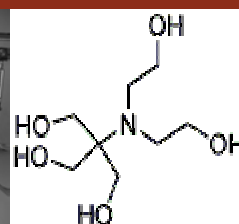
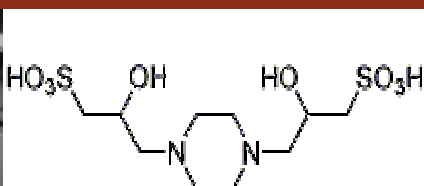
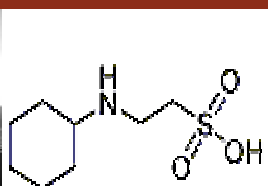
Roman symbols

$[A^-]$	Concentrations of the conjugated base of the acid HA
$A_{s\lambda}^{calc}$	Calculated absorbance of a solution s at a wavelength λ
$d[B]/dpH$	Buffer capacity
C_{HA}	Total concentration of HA acid
C_{si}	Concentration of the i^{th} species in solution s
E^0	Electrode intercept
$E_{1/2}$	Half-wave potential
$E(comp)$	Potential (either $E_{1/2}$ or E_p) in the presence of metal complexes
$E(M)$	Potential (either $E_{1/2}$ or E_p) in the absence of metal complexes
E_{appl}	Applied step potential
E_n^{obs}	Observed electrode potential at the n^{th} data point
E_n^{calc}	Calculated electrode potential at the n^{th} data point
E_p	Peak potential
F	Faraday's constant
$[H^+]$	Proton concentration
$[HA]$	HA acid concentration
Hamilton R-factor, R^H	Statistical parameter generated by ESTA
$[H_T]$	Initial or total proton concentration in the cell
I	Ionic strength
$I(comp)$	Current (either I_d or I_p) in the presence of metal complexes
$I(M)$	Current (either I_d or I_p) in the absence of metal complexes
i_{ac}	Capacitive current
I_{appl}	Observed total current
I_b	Background current
I_d	Limiting diffusion current
I_F	Diffusion reduction current of electrochemically active species recorded at E_{appl}
I_p	Peak current
I_r	Differential reduction current of electrochemically active species recorded at E_{appl}
k	Electrode calibration slope
Ka_n	Ligand protonation constants, where n varies between 1 and 3

K_{ML}	Overall stability constant for the complex ML
K_w	Ionic product of water
l_s	Optical path-length used for the solution s
$[L]$	Free ligand concentration
$[L_T]$	Initial or total ligand concentration in the cell
$[L_T]:[M_T]$	Ligand total to metal total concentrations ratio
$[M]$	Free metal ion concentration
$[M_T]$	Initial or total metal concentration in the cell
n	Number of electrons
n_p	Number of parameters simultaneously optimized
N	Number of experimental points (curves) recorded on a system
$[OH^-]$	Hydroxide concentration
p	Metal stoichiometric coefficient
q	Ligand stoichiometric coefficient
r	Hydroxide stoichiometric coefficient
R	Ideal gas constant
SD	Overall fit of complex formation curves, generated by polarographic program 3D-CFC
T	Temperature
U	ESTA objective function
$w_{1/2}$	Width of the peak at half height
Z_M	Complex formation function generated by ESTA

Greek symbols

χ^2	Chi-squared, statistical parameter generated by the program SUPERQUAD
$\beta_{M_pL_qOH_r}$	Overall stability constants for the metal-ligand complexes
$\beta_{Mx(OH)y}$	Metal hydroxide stability constants
β_{n0l}	Formation constants of $M_nL_0H_l$ species
β_k^H	Cumulative protonation constants
$\varepsilon_{i\lambda}$	Molar absorptivity of the i^{th} species at the wavelength λ
γ	Coefficient describing the steepness of the polarographic wave
λ	Wavelength
σ	Standard deviation



PART I INTRODUCTION

1 INTRODUCTION

1.1 THEORETICAL CHEMICAL SUPPORT OF A pH BUFFER

The concentration of hydrogen ions is of critical importance in biological and chemical systems. Measurement of pH is actually another way of expressing the concentration of hydrogen ions, $[H^+]$, in a solution.

A pH buffer is a solution that resists any change in pH when small amounts of acid or base are added. In the pH range between 3.0 and 11.0, a pH buffer is a mixture of a weak acid (HA) and its conjugated base (A^-). In their presence, the addition or removal of hydrogen ions simply shifts concentrations to restore equilibrium, increasing or decreasing by a small amount the relatively large reservoir of protonated buffer. The efficiency of a buffer is dependent on its buffering capacity (resistance to change in pH by addition of strong acid or base), and its ability to maintain a stable pH upon dilution or addition of neutral salts. The pH of a solution of a weak acid or base may be calculated from the Henderson-Hasselbalch equation [1]:

$$pH = pKa + \log \frac{[conjugated\ base]}{[acid]} \quad (1-1)$$

This equation demonstrates that the buffering action results from the equilibrium properties of an acid and its conjugated base. The pKa of a buffer is that pH where the concentrations of basic and acidic species are equal. In this simple form, this equation is accurate between pH 3.0 and 11.0, being this the pH range of interest [2]. From the Henderson-Hasselbalch equation, an expression for buffer capacity, $d[B]/dpH$, may be deduced. If at some concentration of buffer, C_{HA} , and the sum of the acid and its conjugated base concentrations ($[A^-] + [HA]$) is constant, then the amount of strong acid or base needed to cause a small change in pH is given by the relationship [1, 2]:

$$\frac{d[B]}{dpH} = 2.303 \left(\frac{C_{HA} Ka [H^+]}{(Ka + [H^+])^2} + [H^+] + \frac{Kw}{[H^+]} \right) \quad (1-2)$$

Kw stands for the ionic product of water, and the second and third terms are only significant below pH 3.0 or above pH 11.0, respectively. The maximum buffer capacity occurs when the solution pH is equal to pKa . At that point,

$$\left(\frac{d[B]}{dpH} \right)_{\max} \approx \left(\frac{2.303}{4} \right) C_{HA} \quad (1-3)$$

The buffer capacity of any buffer is dependent on the concentration, C_{HA} , and changes with pH. As result of this variation, in practice, the most effective pH range for a buffer is generally ± 1 unit and is centred on the pK_a of the system [1, 2].

1.1.1 THE IMPORTANCE OF THE pH MAINTENANCE

Hydrogen ion concentrations have important implications in cell metabolism, by affecting the rate of enzymatic reactions and the stability of biological molecules. For example, maintenance of an appropriate pH range in tissue culture media is critical to the growth and viability of all cultured cells. The efficiency of many chemical separations and the rate of many chemical reactions are ruled by the pH of the solution. Buffers can be used to control the rate and yields in organic synthesis. The hydrogen ion concentration is also an important parameter to control in numerous laboratory research techniques such as: electrophoresis, chromatography, and immunoassays. Uncontrolled pH can result in unsuccessful immunoassays since the required protein-protein interactions cannot occur efficiently outside the range of physiological pH. When metal speciation studies are carried out in natural waters, model solutions or culture media for organisms, it is usually desirable to maintain the pH of the solution at a constant level; for this purpose, a pH buffer is also necessary.

Many biological systems generate and consume hydrogen ions as by-products of their cellular reactions, yet respond dramatically to small changes in environmental pH. To maintain a physiologically relevant pH (pH = 6.0-8.5) under such dynamic conditions, *in vitro* biological systems must be stabilized by the incorporation of buffers that undergo reversible protonation. Biochemical reactions may occur optimally at pH values ranging from 4.5 to 9.7. Many early buffers were not suitable for biological applications because the pH of the solutions depended upon the concentration of the ionic components and the temperature of the solution. Moreover, the pK_a values of many of these buffers were outside physiological pH ranges. For illustration, many early biological buffers and their associated pK_a values are summarized in Table 1-1 [3].

Due to inefficacy, undesired reactivity or toxicity, these were inappropriate buffers. Phosphate has poor buffering capacity above pH 7.5 and is an active participant in many biochemical processes, being an activator, inhibitor and/or a metabolite in many biological systems. Phosphates also demonstrate complexing capabilities with divalent and polyvalent cations and can therefore inhibit a series of metal ion-dependent biochemical reactions [3]. Citric and succinic acids form complexes with various cations [3].

Table 1-1 Common biological buffers and their pK_a values at 25.0°C and 0.1M ionic strength

pKa and protonation reaction	pKa₁	pKa₂	pKa₃
Buffer	H + L $\rightleftharpoons$ HL	HL + L $\rightleftharpoons$ H₂L	H₂L + L $\rightleftharpoons$ H₃L
Phosphoric acid	1.90	6.71	11.52
Citric acid	2.90	4.35	5.64
Succinic acid	3.99	5.24	
Acetic acid	4.56		
Imidazole	7.00		
TRIS	8.10		
Glycylglycine	8.09		
Boric acid	8.98		

Imidazole is used to prepare buffers in the pH range of 6.2-7.8 at 25°C and is also a chelator of various divalent cations [3]. 2-Amino-2-(hydroxymethyl)-1,3-propanediol (TRIS), which was used for speciation studies [4-7], is not a very efficient buffer below pH 7.5 and is a potentially reactive primary amine which often acts as an inhibitor. It has an appreciable solubility in organic solvents which permits it to penetrate biological membranes and it has a very high pH temperature coefficient [8]. Glycylglycine is an expensive buffer and only works well above pH 8.0. Borate buffers complex with a wide variety of important respiratory metabolites and other organic compounds as well [8]. Nevertheless many side effects cannot be predicted and buffers may uncouple or inhibit or modify reactions by mechanisms not yet understood.

Clearly, a buffer which is adequate or even better in one system may not be very good in another system. For these reasons, the selection of an appropriate buffer will always depend on extensive testing. Consequently, it is important that a number of buffers are available for each pH range.

1.1.2 GOOD'S BUFFERS

In 1966, Good *et al.* described twelve buffers [8] which were useful for most common biological applications. Most of these buffers are zwitterionic, containing both positive and negative ionisable groups. Secondary and tertiary amines provided the positive charges, while sulfonic and carboxylic acid groups provided the negative charges. On designing these buffers several criteria were expected [8]: 1) pK_a values should be between 6 and 8, since this was the region where less buffers were available and most biological reactions take place; 2) the buffer should have maximum water

solubility, to allow the use of concentrated stock solutions and minimum lipid solubility, making it impermeable to membranes; 3) there should be minimal effect of temperature, ionic strength or buffer concentration on the pK_a ; 4) the buffers should not form complexes with cations, or, if they do, the complexes should be soluble, and the binding constants determined; 5) the buffers should be stable, not metabolized, and should not act as enzyme inhibitors or substrate analogues; 6) they should not absorb light above 240nm, and particularly not in any region that would be used in spectrophotometric assays and finally 7) they should be easy to prepare and inexpensive.

Zwitterionic N-substituted aminosulfonic acids seemed to meet most of the criteria. But none of the buffers meet all of the criteria listed above; none of the buffers were truly inert in biological systems. The determination that propane sultone, the precursor compound required for the synthesis of some zwitterionic buffers is carcinogenic and hazardous to use, led to the synthesis of hydroxyl derivatives of the buffers [9]. These compounds were found to be compatible with a number of biological systems while expressing better chemical stability and improved solubility over the earlier Good's buffers.

All of Good's buffers (often called "Good" buffers) are now commercially available and at least two major chemical suppliers, Sigma and Fluka, devotes a specific section of their catalogue to biological buffers [10, 11]. More recently, Thiel *et al.* developed new buffers with butane containing side chains: 4-(N-Morpholino)butanesulfonic acid (MOBS), N-tris(Hydroxymethyl)methyl-4-aminobutanesulfonic acid (TABS), N-(2-Hydroxyethyl)piperazine-N'-(4-buthanesulfonic acid) (HEPBS), and 4-(Cyclohexylamino)-1-buthanesulfonic acid (CABS), extending the useful buffering range of buffers into the more alkaline range [12]. Figure 1-1 shows the useful pH ranges [10], pK_a values [3], and the acronyms by which Good buffers and some other useful biological buffers are known [10].

Studies involving metal ions in solution which require pH control are inevitably subject to the possibility of buffer interferences as a result of complexation. One of Good and co- workers criteria in generating a series of buffers for use in biological studies was, as stated above, the prevention of metal complexation, or in case of occurring, the stability constants of the soluble complexes formed should be known. Good buffers are presently used as a matter of routine in biochemical, biological and speciation studies. As examples, Blatny *et al.* [13] and Haskins *et al.* [14] used MES for total quantification of iron and europium, respectively, and Parthasarathy and Buffle [15] used MES for copper speciation studies.

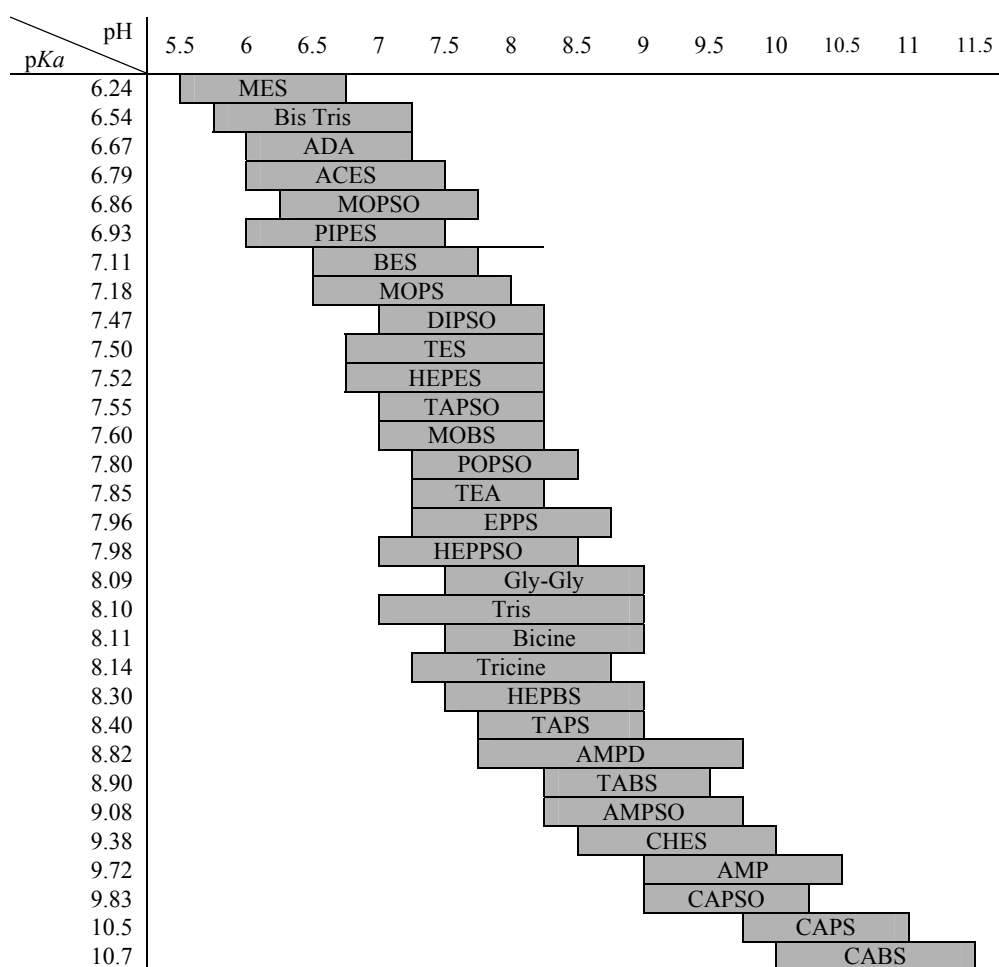


Figure 1-1 Useful pH ranges of some selected biological buffers [10], pKa values [3], and the acronyms by which Good buffers and some other useful biological buffers are known [10]

2-(N-Morpholino)ethanesulfonic acid; 4-Morpholineethanesulfonic acid (MES), 3-Morpholino-2-hydroxypropanesulfonic acid (MOPSO) and 3-(N-Morpholino)propanesulfonic acid (MOPS) have also been used for the separation of organic and inorganic compounds [16-18] and determination of organic compounds by cathodic stripping voltammetry [19-21]. There are also reports of the use of MES, MOPSO, TRIS and MOPS in biochemical studies [22-24].

4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and 1,4-Piperazinediethanesulfonic acid (PIPES) have already been used for zinc [25] and iron [26, 27] speciation studies, respectively. HEPES was used in organic copper speciation studies in estuarine waters by cathodic stripping voltammetry [28]. TRIS, 4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS), and PIPES were used in speciation studies of iron in seawater, by cathodic stripping voltammetry. In the studies performed, the possible interference of these buffers was taken into account [7]. Copper complexation by the 1,4-benzodiazepines, an extensively studied class of psychotherapeutic drugs, was investigated using differential pulse polarography and cyclic voltammetry at a mercury electrode in 0.10M KNO₃

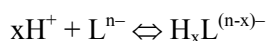
and $\text{pH} = 7.0 \pm 0.1$ by addition of PIPES [29].

In many situations, all these and other biological buffers are used under the assumption that they undergo little if any interaction with biological or environmental important metal ions. Yet increasing reports of interferences arising from the use of Good buffers can be found on literature. Several studies focusing on the toxicity of copper have used TRIS as a chelator [4, 5] as well as a pH stabilizer. However, in addition to complexing copper [30], it was shown that TRIS interfered with the growth of several phytoplankton [31] and sustained active bacterial growth in nonaxenic microalgal cultures [32]. The influence of 3-(*N,N*-Bis[2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid (DIPSO) and HEPES on the toxicity of copper(II) to the marine dinoflagellate, *Amphidinium carterae*, was investigated. Neither of the buffers was toxic to *Amphidinium carterae*. HEPES did not form metal complexes but increased copper toxicity on opposition to DIPSO that forms strong complexes with copper and decreased its toxicity [33]. Also the influence of 4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid) (HEPPSO) and Piperazine-1,4-bis(2-hydroxypropanesulfonic acid) (POPSO), at pH 8.0, on the biological response of *Amphidinium carterae* Hulburt, was investigated [34]. The study demonstrated that POPSO enhanced copper uptake and toxicity and HEPPSO reduced uptake and toxicity of copper because of its enhanced binding capacity compared to POPSO [34], as it was demonstrated previously [35, 36]. A recent study of the influence of HEPES on the biological response of marine algae demonstrated that HEPES can control the uptake of trace metals by algae and the production of organic ligands [37].

Stellwagen *et al.* investigated the interactions of DNA with various neutral pH amine-based buffers, MOPS, HEPES, *N,N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES) and *N*-[Tris(hydroxymethyl)methyl]glycine (Tricine), by free solution capillary electrophoresis. The distorted peaks in the electropherograms indicated that buffer-DNA complexes were formed [38]. Particular problems have also been identified in protein assays and were attributed to the complexation of the Good buffer with copper. Llew and Rebel [39] showed that low concentrations (0.2 to 5mM) of HEPPSO, EPPS, *N,N*-Bis(2-hydroxyethyl)glycine (Bicine), HEPES, DIPSO and 3-[(1,1-Dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO) interfered with protein determination, using Lowry method [40]. Smith *et al.* have described a modification of the Lowry method using bicinchonic acid (BCA method) [41] that does not interfere with protein assays when low concentrations of these buffers are used, except for AMPSO [39].

1.2 METAL ION-BUFFER COMPLEXATION STUDIES

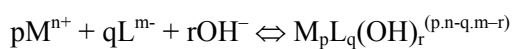
Extensive information about the stability constants of metal ions complexation with all biological buffers is not found in the literature. A careful search in the computerized database of critical constants developed by Smith, Motekaitis, and Martell, The National Institute of Standards and Technology (NIST) Standard Reference Database [3] was done and the information about structure, pK_a and stability constants with several metals is summarized in Table 1-2 to Table 1-7. The tables were organized by families structures of the buffers, since many of the buffers only differ in the length of the alkane group side chain. The ligand protonation constants presented in the tables are defined as:



with

$$Ka = \frac{[H_xL^{(n-x)-}]}{[H^+]^x [L^{n-}]} \quad (1-4)$$

and the formation constants for the metal complexes are defined as:



with

$$\beta_{pqr} = \frac{[M_pL_q(OH)_r^{(p.n-q.m-r)}]}{[M^{n+}]^p [L^{m-}]^q [OH^-]^r} \quad (1-5)$$

in the case of the formation of complexes involving the protonated form of a ligand, e.g. MLH , the OH^- should be replaced by H^+ .

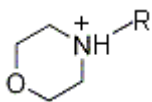
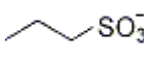
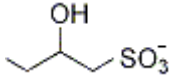
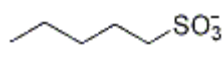
Stability constants of metal ion complexes in the literature have frequently been published by several investigators; values that differ by one, two or even more orders of magnitude have been reported. For instance, Anwar *et al.* published a $\log K_{CuL}$ for MOPS of 4.00 [42] and in the same year Soares *et al.* demonstrated that MOPS does not bind copper [43]. These variations can have several reasons including impure ligand, poor experimental performance, inaccurate measurements and erroneous calculations [44].

Smith and Martell developed a compilation of critically selected stability constants (NIST Database) such that every complex formation reaction is indicated by one and only one equilibrium constant for a specific set of conditions. A list of minimum criteria was created in order to develop a

systematic way of evaluating new data on stability constants [45]. One of the most important factors regards experimental control, and the care with which the reaction conditions and measurements were made and clearly explained. An adequate description of the measurements, apparatus and its calibration is also necessary. Also the lack of information on the purity of the ligand and failure to define the equilibrium quotients that are reported is enough to disable the inclusion of the result in the database. In Table 1-2 to Table 1-7, metal-buffers stability constants, that fulfilled the criteria established by Martell and Smith [45], were included.

In Table 1-2, the results for a series of N-substituted aminosulfonic acids (MES, MOPSO, MOPS and MOBS) with a morpholinic ring pH buffers are compiled. NIST Database [3] reports ligand protonation constants for all buffers, unless MOBS. Other information related with metal-buffer complexation was not included because do not meet criteria for publication [3]. Additionally, studies of the interaction of MES, MOPSO and MOPS with copper, lead, cadmium and zinc [43, 46] have shown that MES, MOPSO and MOPS do not form metal complexes with copper and zinc. However MOPSO and MOPS modify the reversibility of copper and zinc electrochemical response [43]. No metal complexation was also observed between lead and cadmium in the presence of MES, MOPSO or MOPS [46].

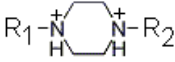
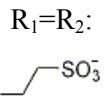
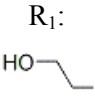
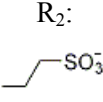
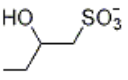
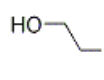
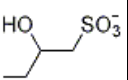
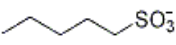
Table 1-2 Structure, pK_a and stability constants values (as $\log \beta$) of morpholinic family buffers. Unless stated, values were compiled from NIST Database [3].

Morpholinic Family	Name	MES	MOPSO	MOPS	MOBS
	R Structure				
	pK_a	6.24	6.86	7.184	(7.6)
	Cu	NEC	NEC*	NEC*	NI
	Pb	NEC	NEC	NEC	NI
	Cd	NEC	NEC	NEC	NI
	Zn	NEC	NEC	NEC*	NI
	Ni		*	*	NI
	Co			*	NI

NEC – no evidence of complex formation; NI – Not Included in NIST Database; * – published data do not meet criteria for critical selection; the protonation constant in brackets is an indicative value

The results of the complexation studies of a series of N-substituted aminosulfonic acids with piperazinic ring: PIPES, HEPES, POPSO, EPPS, HEPPSO and HEPBS and the metal ions copper, lead, cadmium, zinc, nickel and cobalt are presented in Table 1-3. PIPES does not form metal complexes with copper [47], cadmium and zinc [48]. HEPES does not bind cadmium or zinc [48], but forms complexes with copper [49]. A slight complexation between lead and PIPES [48] and HEPES [48] was observed. A change in metal electrochemical reversibility due to influence of these buffers was also observed [48]. No complexation was detected in systems containing EPPS and cadmium or zinc [50]. HEPPSO does not bind cadmium [48] but forms complexes with lead and changes the reversibility of the metal electrochemical response [48]. For HEPBS, no information is available up to now.

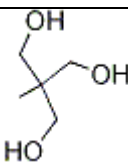
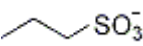
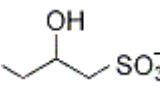
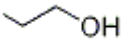
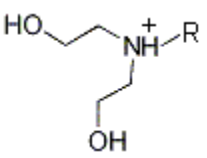
Table 1-3 Structure, pK_a and stability constants values (as $\log \beta$) of piperazinic family buffers. Unless stated, values were compiled from NIST Database [3].

Piperazinic Family	Name	PIPES	HEPES	POPSO	EPPS	HEPPSO	HEPBS
	R₁ and R₂ Structure		R ₁ :  R ₂ : 	R ₁ =R ₂ : 	R ₁ :  R ₂ : 	R ₁ :  R ₂ : 	R ₁ :  R ₂ : 
	pK_a	6.93	7.52	(7.8)	7.957 ^b	7.98	(8.3)
	Cu	* NEC	ML=3.22 ^a	NI	ML=2.90 ^c	*	NI
	Pb	SC	SC	NI	ML=3.1 ^c	ML=2.56	NI
	Cd	NEC	NEC	NI	NEC	NEC	NI
	Zn	*NEC	NEC	NI	NEC	*	NI
	Ni	*		NI		*	NI
	Co	*		NI		*	NI

* – published data do not meet criteria for critical selection; NEC – no evidence of complex formation; SC – slight complexation; NI – Not Included in NIST Database; ^a - value from reference [49]; ^b - ionic strength 0.0M; ^c - Values obtained from reference [50]

In Table 1-4, the complexation results for the (2-hydroxyethyl)amine family buffers (2-Bis(2-hydroxyethyl)amino-2-(hydroxymethyl)-1,3-propanediol – BIS-TRIS, BES, DIPSO and Triethanolamine – TEA) are summarized. Table 1-4 shows that reliable information regarding the complexation of BIS-TRIS and TEA with all the metals, copper, lead, cadmium, zinc, nickel and cobalt can be found. No reliable information about the possible interaction of BES and DIPSO with these metals was found in NIST Database; the published information of the systems $\text{Cu}-(\text{BES})_x-(\text{OH})_y$, $\text{Co}-(\text{BES})_x-(\text{OH})_y$, $\text{Cu}-(\text{DIPSO})_x-(\text{OH})_y$, $\text{Zn}-(\text{DIPSO})_x-(\text{OH})_y$, $\text{Ni}-(\text{DIPSO})_x-(\text{OH})_y$ and $\text{Co}-(\text{DIPSO})_x-(\text{OH})_y$ does not meet criteria for critical selection.

Table 1-4 Structure, pK_a and stability constants values, as $\log \beta$, of bis(2-hydroxyethyl)amine family buffers. Values were compiled from NIST Database [3].

Bis(2-hydroxyethyl)amine Family	Name	BIS-TRIS	BES	DIPSO	TEA
	Structure				
	pK_a	6.54	7.11	7.6 ^b	7.85
	Cu	ML=5.27 ^a	*	*	ML=4.07 ML(OH)=(12.44) ML ₂ (OH)=14.43 M ₂ L ₂ (OH) ₂ =28.08 ^c
	Pb	ML=4.32 ^a			ML=3.39 ML ₂ =5.86 ML(OH) ₂ =16.44 M ₂ L ₂ (OH) ₂ =15.67
	Cd	ML=2.47 ^a			ML=2.70 ML ₂ =4.60 ML ₃ =5.21
	Zn	ML=2.38 ^a		*	ML=2.05 ML ₂ =3.28
	Ni	ML=3.59 ^a		*	ML=2.76 ML ₂ =3.6 M ₂ L ₂ (OH) ₂ =18.28 ^c
	Co	ML=1.78 ^a	*	*	ML=(2.25)

^a - ionic strength equal to 1.0M; ^b - ionic strength equal to 0.0M; ^c - ionic strength equal to 0.5M; * – published data do not meet criteria for critical selection; the stability constant values in brackets are indicative values

In Table 1-5, buffers derivated from the TRIS were included. The Table 1-4 and Table 1-5 evidence that the recorded stability constants for some $M-(TEA)_x-(OH)_y$ and all $M-(TES)_x-(OH)_y$ systems studied are not completely trustworthy, since are indicative values. In addition, the published data of the possible complexation of 2-Hydroxy-3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid (TAPSO) with copper, nickel and cobalt, as well as the [(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]-1-propanesulfonic acid (TAPS) protonation constant and its interaction with copper and cobalt found by Martell and Smith in the literature up to 2004 did not meet criteria for inclusion in NIST Database [3].

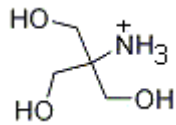
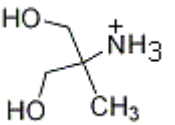
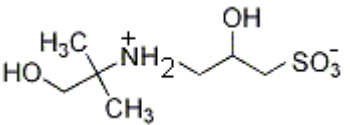
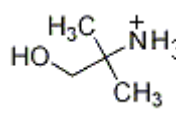
Table 1-5 Structure, pK_a and stability constants values (as $\log \beta$) of TRIS family buffers. Values were compiled from NIST Database [3].

TRIS Family	Name	TES	TAPSO	TAPS	TABS
	Structure				
	pK_a	7.50	7.60	*	(8.9)
	Cu	ML=(3.90) ML(OH)=(6.62)	*	*	NI
	Pb				NI
	Cd				NI
	Zn	ML=(2.08)			NI
	Ni	ML=(3.35) ^a ML(OH)=(8.58)	*		NI
	Co	ML=(2.07)	*	*	NI

* – published data do not meet criteria for critical selection; ^a – Values recorded at 20°C; NI – Not Included in NIST Database; the stability constant values in brackets are indicative values

The information regarding complexation studies between TRIS, 2-Amino-2-methyl-1,3-propanediol (AMPD), AMPSO and 2-Amino-2-methyl-1-propanediol (AMP), and the metal ions copper, lead, cadmium, zinc, nickel and cobalt are summarized in Table 1-6. This table evidences that TRIS complexes with several metal ions (Cu, Cd, Zn, Ni and Co). For the other buffers (AMPD, AMPSO and AMP), the published metal complexation studies do not meet criteria for critical selection for being included in NIST Database [3].

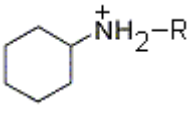
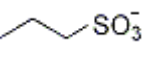
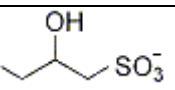
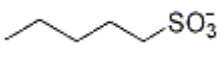
Table 1-6 Structure, pK_a and stability constants values (as $\log \beta$) of some biological buffers. Values were compiled from NIST Database [3].

Name	TRIS	AMPD	AMPSO	AMP
Structure				
pK_a	8.10	8.82	9.08	9.72
Cu	ML=4.05 ML ₂ =7.6 ML ₃ =11.1 ML ₄ =14.1	*	*	*
Pb				
Cd	ML=1.88	*		*
Zn	ML=2.27		*	
Ni	ML=2.63 ML ₂ =4.6	*	*	*
Co	ML=1.73		*	

* – published data do not meet criteria for critical selection

Available information for buffers belonging to cyclohexylamino family was included in Table 1-7. For this family, only protonation constants values for 2-(Cyclohexylamino)ethanesulfonic acid (CHES), 3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO) and 3-(Cyclohexylamino)-1-propanesulfonic acid (CAPS) are available.

Table 1-7 Structure, pK_a and stability constants values (as $\log \beta$) of Cyclohexylamino family buffers. Values were compiled from NIST Database [3].

Cyclohexylamino Family	Name	CHES	CAPSO	CAPS	CABS
	Structure				
	pK_a	9.38	9.825 ^a	10.499 ^a	(10.7)
	Cu				NI
	Pb				NI
	Cd				NI
	Zn				NI
	Ni				NI
	Co				NI

^a - ionic strength 0.0M; NI – Not Included in NIST Database; the protonation constant in brackets is an indicative value

Based in the meticulously bibliographic search performed, it is possible to say that complete information about the metal ions complexation properties of biological buffers is far from being available. The analysis of Table 1-3 and Table 1-4 points out that several studies were already performed to test the metal complexation properties of morpholinic and piperazinic buffers, but scarce information relatively to the other families, was reported.

In a general way, morpholinic and piperazinic buffers are none or weaker complexing agents; Table 1-3 and Table 1-4 show several non complexing buffers. On the other hand, the analyses of the structures of the buffers presented in Table 1-4 to Table 1-6, as well as some metal stability constants mentioned in these tables, clearly indicate that buffers belonging to these families are important metal complexing agents. These compounds are good complexing agents because they all contain hydroxyl groups in addition to amine nitrogen. Therefore, metal complexes of these buffers are formed in biochemical / environmental systems containing metal ions when these compounds are used to control the pH.

1.3 METHODOLOGY

Several methods are available for determining complex equilibrium constants. Among others, potentiometry, spectrophotometry, specific metal ion electrodes, nuclear magnetic resonance spectroscopy (NMR), electron spin resonance (ESR) and polarography have been used [45, 51]. In the top of applied techniques list is potentiometry with about 80% of the stability constants in the literature. Second on the list is spectrophotometry, followed by the use of specific metal ion electrodes. These three techniques are responsible by about ninety five per cent of the stability constant data in the literature [45].

The use of potentiometry is explained by the fact that most ligands have considerable basicity and control the hydrogen ion concentration as well as the metal ion concentration. Therefore, determination of the hydrogen ion concentration provides a measure of the concentration of the ligand at equilibrium, with or without the metal ion under investigation, providing enough data to determine the affinity constant between the metal ion and the ligand. In this type of measurement, the glass electrode is very convenient since it is a single ion electrode whose potential is sensitive to the concentration of the free hydrogen ion. In general, if the pH at equilibrium is between 2 and 12 the hydrogen ion concentration can be determined accurately enough to carry out the stability constant calculations [45]. The change in the hydrogen concentration must be caused mainly by the formation of complexes, which means that the employed concentrations of the metal ion are in the region of 10^{-3} – 10^{-2} M. Additional information of this technique can be found in Part II, Chapter 2 and Part V, Chapter 10.

In recent years, a number of specific metal ion electrodes have been developed, which allow the direct measurement of the concentration of the metal ion. Thus, it is possible to measure directly the concentrations of the alkali metal ions and the alkaline earth metal ions and a number of others ions by determining the potential of the electrode that is specific for that ion and consequently determine the binding constants of a (metal ion)–(ligand) system. For instance, the system Cd–(N-(2-hydroxyethyl)iminodiacetic acid) was studied by ion selective electrode of cadmium [52]. The use of these type of electrodes is generally restricted to total ion concentrations above 10^{-6} M, depending on the ion measured [53, 54].

Potentiometry does not provide microscopic information involving identification of protonation and metal coordination sites on a ligand. For such information, spectroscopic measurements (e.g. NMR) or spectrophotometric absorbance methods studies are needed [45, 51]. Absorbance is the quantity measured spectrophotometrically, which is related with the extinction coefficient of the species formed and the concentration of each species. The ideal situation is when there is only one absorbing species or when the absorbing species absorb at different wavelengths and are easily

separated, but in most cases it exist two or more species with overlapping absorption bands. In these situations, all absorbing species must be taken into account, which may include the metal ion, the various complexes of the metal ion, and the absorbance of the free ligand. The difficulties in obtaining the stability constants values depends on whether the value(s) for the extinction coefficient(s) can be obtained for each species or have to be calculated. The concentrations that are possible to be used depend on the extinction coefficient of the metal and ligand. For absorbing ligand, the range of concentrations varies between 10^{-6} and 10^{-3} M.

NMR and ESR are two magnetic resonance techniques that have been applied to binding studies. Valuable structural information, much of it unavailable by other methods, has been obtained about complexes in solution, primarily from chemical shift measurements of the complexed species. ESR is applicable to paramagnetic substances, namely those that possess an unpaired electron, and therefore is less widely applicable to the measurement of binding constants than is NMR [55]. These two last techniques were used to characterize the complex formation of copper(II) with triethanolamine in aqueous solution [56]; the stability constants were determined by ESR and ^1H NMR was used to study chelate effects. Several reports can be found in literature of solution equilibria using various spectroscopic techniques [57-61].

Polarography, as well as all the other voltammetric techniques, is a dynamic and in principle non-equilibrium analytical technique. Polarographic measurements result in a signal, whose position and intensity depends on the solution composition; it is based in the shift and / or the intensity of a polarographic signal that stability constants can be evaluated. The use of polarography is increasing due to its specific advantages for the study of complexes at low pH and low total metal concentration ($10^{-6} - 10^{-5}$ M), resulting in its application in the study of complexes of such very acidic metal ions as Bi^{3+} [62]. Polarography can also be applied to the more complete study of complexes of other metal ions with low solubility and when dealing with easily hydrolysable ions to form hydroxide precipitates [63]. Complementary information about this technique can be found in Part II, Chapter 2 and 3.

Among the others methods for measuring complex equilibria, fluorimetry is the technique that uses lower amount of ligand, concentrations as low as 10^{-7}M . Several types of fluorescence measurements can be used in the study of binding constants. Fluorescence polarization is a measure of rotational relaxation time, so if a small molecule binds to a large molecule its fluorescence polarization will change to reflect the relaxation time of the large molecule. Most applications of fluorimetry to binding have made use of fluorescence intensity measurements [55]. Hays *et al.* used this technique to determine the copper(II) conditional stability constants and ligand concentrations of fulvic acid [64]; Rodriguez-Caceres *et al.* used fluorescence to investigate the metal-ligand complexes of mono- and di-substituted naphthalene derivatives [65].

1.4 THESIS AIMS

The central aim of this thesis was to contribute with additional information of which pairs buffer-metal ions exhibit complexation and determine their global stability constants.

The selected amino-substituted pH buffers were: TAPSO, TAPS and AMPSO. These buffers cover a large pH-range which embraces the range of pH that occurs in the environmental and biological systems. Due to their biological / biochemical and/or environmental importance, six divalent metal ions were selected: Cd, Co, Cu, Ni, Pb and Zn.

The study of the chemical binding between biological buffers and metals ions was performed using three experimental methods: polarography, differential pulse and/or direct current polarography (DPP and/or DCP); glass electrode potentiometry (GEP) and UV-Vis spectroscopy. The combination of these techniques proved to be a powerful tool to characterise properly the several (metal ion)–(buffer) systems. The combination of potentiometry and polarography allowed covering ligand total to metal total concentrations ratios ($[L_T]:[M_T]$) of various orders of magnitude. Between 3, by GEP (copper) and 799 (zinc), by DCP and total metal ion concentration between 10^{-6} and 10^{-3} M. As far as we know, no other combination of techniques should be able to cover a so wide range of total metal concentration and $[L_T]:[M_T]$ ratios with these type of ligands. It is important to note that the biological buffers under study are basic ligands ($7.55 < pK_a < 9.05$) with no double bonds, so fluorescence, which uses low concentration of ligand, could not be applied for these systems because the biological buffers do not absorb.

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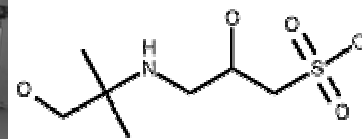
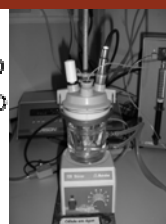
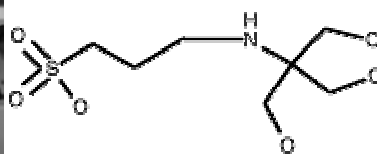
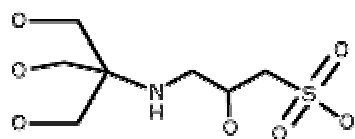
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PART II MODELLING OF Cu-(BUFFER)_x-(OH)_y SYSTEMS

2 CHALLENGES IN MODELLING AND OPTIMISATION OF STABILITY CONSTANTS IN THE STUDY OF METAL COMPLEXES WITH MONOPROTONATED LIGANDS. A GLASS ELECTRODE POTENTIOMETRIC AND POLAROGRAPHIC STUDY OF A $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ SYSTEM¹

ABSTRACT

The behaviour of solutions containing TAPSO and copper(II) was studied by two analytical techniques, DCP and GEP, at fixed total TAPSO to total copper(II) concentration ratios and various pH values, at 25°C and ionic strength 0.1 M KNO_3 . DCP and GEP, when used independently, were not able to provide a final metal-ligand model. Combined interpretation of data from DCP and GEP indicated the formation of six main species, CuL^+ , $\text{CuL}(\text{OH})$, $\text{CuL}(\text{OH})_2^-$, CuL_2 , $\text{CuL}_2(\text{OH})^-$ and $\text{CuL}_2(\text{OH})_2^{2-}$ for which stability constants (as $\log \beta$) were found to be 4.41, 11.43, 17.55, 8.08, 14.3 and 20.3, respectively. Five of these complexes, $\text{CuL}(\text{OH})$, $\text{CuL}(\text{OH})_2^-$, CuL_2 , $\text{CuL}_2(\text{OH})^-$, and $\text{CuL}_2(\text{OH})_2^{2-}$ are reported for the first time.

2.1 INTRODUCTION

More than thirty years ago, Good and co-workers published a classic paper [1] in which they proposed twelve buffer systems to supplement or replace TRIS and phosphate buffers commonly used by biologists and biochemists. In two subsequent papers, eight additional buffers were proposed [2, 3].

Studies involving metal ions in solution, which require pH control, are inevitably subject to the possibility of buffer interferences as a result of complexation. In generating a series of more than twenty buffers for use in biological studies, Good and co-workers listed the prevention of metal complexation as one of their desired goals. Good's buffers (often called "Good" buffers) are presently used routinely in biochemical and speciation studies under the assumption that they undergo little if any interaction with biologically (or environmentally) important metal ions. However, there has been an increase in reports on buffer interferences when most of Good's buffers were used in the presence of metal ions [4, 5]; many conflicting data and conclusions that were reported by investigators studying identical metal-cation protein systems (at the same pH in carefully executed experiments) might be due in part to the failure to include the presence of metal-buffer complexes [6]. These compounds (Good's buffers) contain hydroxyl groups in addition to a primary nitrogen donor atom.

¹ Analytica Chimica Acta 493 (2003) 105-119

Therefore, one might expect that metal complexes of these buffers would form in biochemical/environmental systems containing metal ions when these compounds are used to control the pH.

Until now, with the exception of a few reports regarding studies of the ternary complexes of biological importance [7-9], an exhaustive speciation study of $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ system has never been performed. In this context, we think that the study of the interactions of TAPSO with copper(II) may furnish a further contribution related to the complexation properties of this buffer when used in the study of environmental and biological systems.

In this work, we report the stability constants for species formed in the $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ system at 25°C and ionic strength 0.1 M KNO_3 . Two electrochemical techniques, DCP and GEP, were employed. Five new complexes of copper with TAPSO were identified. Experiments by alternating current polarography (ACP) were also carried out to check if this buffer adsorbs at the mercury working electrode surface under the experimental conditions used.

2.2 EXPERIMENTAL

2.2.1 MATERIALS AND REAGENTS

All glassware was cleaned with 0.5 M HNO_3 , and rinsed several times with distilled water. Deionised water with resistivity $> 4 \text{ M}\Omega\cdot\text{cm}$ was used throughout the experiments.

The solid potassium hydroxide was obtained from Merck (Darmstadt, Germany). A stock solution of about 0.1 M KOH (prepared by dissolving of KOH(s)) was standardised against potassium hydrogen phthalate weakly by potentiometric titration. The equivalence point was determined by Gran plot method [10]. This method also allowed to verify if the strong base solution was contaminated with carbonate. A stock solution of about 0.1 M nitric acid (prepared from a concentrated acid (Merck)) was standardised potentiometrically against the standardised solution of potassium hydroxide. The KNO_3 was supplied by Merck and was used as a background electrolyte to adjust the ionic strength of samples. All measurements reported in this work were performed at 25 °C on solutions adjusted to ionic strength 0.1 M ($\text{HNO}_3 / \text{KNO}_3$). The ligand TAPSO (99.8%) was purchased from Sigma-Aldrich (St Louis, Missouri, USA) and used as received. The stock solution of copper, $5 \times 10^{-2} \text{ M}$, was prepared from the analytical grade salt ($\text{Cu}(\text{NO}_3)_2$, Merck). It was acidified with nitric acid to a pH of 2.5 and the ionic strength was adjusted to 0.1 M. In potentiometry, a standard solution of copper ($5.0 \times 10^{-2} \text{ M}$), purchased from Merck, was used. High purity nitrogen was used for deaeration of the sample solutions, *i.e.* to remove the dissolved oxygen from the solutions.

2.2.2 APPARATUS

All experiments were carried out in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer, and thermostatted at 25.0 ± 0.1 °C using a water bath.

2.2.2.1 POLAROGRAPHY

Polarographic measurements were performed using a Model 663 VA stand (Metrohm) equipped with a multimode electrode (Metrohm, Model 6.1246.020) as a working electrode, used in the dropping mercury electrode mode. A silver/silver chloride (3M KCl) and a glassy carbon were used as reference and counter electrode, respectively (both from Metrohm). The VA stand was attached to an Autolab (Eco Chemie, Utrecht, The Netherlands) system controlled by a personal computer. The pH measurements were conducted with a 713 pH meter (Metrohm), with a sensitivity of ± 0.1 mV (± 0.001 pH units), with a silver/silver chloride reference electrode (Metrohm) and a glass electrode (Metrohm).

2.2.2.2 POTENTIOMETRY

The potentiometric titrations were performed with a PC-controlled system assembled with a Crison MicroPH 2002 meter, a Crison MicroBU 2030 micro burette, a Philips GAH 110 glass electrode and a Orion 90-02-00 (double junction) reference electrode with the outer chamber filled with 0.1 M KNO_3 . Automatic acquisition of data was done using a home-made program, COPOTISY.

2.2.3 PROCEDURE

2.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

The calibration of the glass electrode was accomplished by addition of the standardised solution of potassium hydroxide to the standardised solution of nitric acid (both solutions adjusted to ionic strength of 0.1 M). From this potentiometric titration, the E° and the response slope were established and used to calculate the pH of the sample during polarographic titration.

2.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

pH buffer adsorption studies were performed by ACP using an amplitude of sinusoidal alternating

current of 5 mV, a frequency of 75 Hz and a scan rate of 2.5mV/s; the capacitive current (i_{ac}) was measured at a phase angle of 90°. This study was done for buffer concentrations in a range from 8.0×10^{-4} to 1.5×10^{-2} M and at three different pH values: 5.55, 7.55 and 9.55. These pH values were chosen because they correspond to experimental conditions where TAPSO occurs as HL (100%), HL and L⁻ (50% of each) and L⁻ (100%), respectively. The potential range scanned in the experiments was from 0.2 to -1.2 V *versus* Ag/AgCl(s), 3 M KCl.

2.2.3.3 DIRECT CURRENT AND DIFFERENTIAL PULSE POLAROGRAPHY

The study of the electrochemical behaviour of Cu-(TAPSO)_x-(OH)_y system was performed by DCP and DPP. For both techniques, a drop time of 1s and a step potential of 4 mV were used. A modulation time of 20 ms and modulation amplitude of 50mV were used in DPP mode. To make sure that the vessel was not contaminated, a first scan was carried out on a deaerated background solution, 20.00 mL of 0.1 M of KNO₃. Then, a required amount of copper stock solution was added and three polarograms were recorded after the solution has been deaerated. Several total ligand to total metal ion concentration ratios ([L_T]:[Cu_T]) were used. The total copper concentration was 5.0×10^{-5} , 2.0×10^{-5} , and 2.4×10^{-5} M for [L_T]:[Cu_T] of 100, 50, and 45, respectively. For each independent titration, a required amount of solid ligand was added to the vessel containing the metal ion solution. After deaeration, a set of three polarograms were recorded. With additions of standardised base solution, the pH was varied in pH steps of 0.1 units between pH 3.0 and 10.0. After each KOH addition, a new polarogram was recorded; in this way, about 60 to 70 polarograms were obtained. The equilibrium of the metal-ligand solutions was tested and it was reached in a few minutes.

2.2.3.4 GLASS ELECTRODE POTENTIOMETRY

For potentiometry, the calibration of the electrodes was done before each titration using two buffer solutions with pHs 6.865 and 4.008, with ionic strength adjusted to 0.1M [11]. The plot of potential *versus* pH gave the working slope and the intercept so that the pH could be read as $-\log [H^+]$. Potentiometry was firstly used to determine the protonation constants of the buffer at 25°C and an ionic strength of 0.1 M KNO₃. To accomplish this, several titrations were performed on different TAPSO solutions containing 1.0×10^{-3} or 5.0×10^{-3} M TAPSO in 0.1 M KNO₃ adjusted to pH 2.5 – 3.0, with KOH 0.01M. Several potentiometric titrations of different metal to ligand ratios set to 1:1, 1:2, 1:3, and 1:4 ([Cu²⁺] = 9.44×10^{-4} M) and 2:1 ([Cu²⁺] = 1.89×10^{-3} M) in 0.1M KNO₃ adjusted to pH 2.5 – 3.0, with KOH 0.01M were carried out in order to establish the model and determine the complex formation constants of Cu-(TAPSO)_x-(OH)_y system.

2.2.4 DATA TREATMENT

The refinement of the polarographic data was performed using a speciation method recently developed [12, 13]; this method uses mass-balance equations written for a labile and reversible metal-ligand system studied at a fixed total ligand [L_T] to total metal [M_T] concentration ratio and varied pH. The model is refined by comparing experimental

$$\left(E(M) - E(comp) \right)_{pH(i)} - \frac{RT}{nF} \ln \left(\frac{I(comp)}{I(M)} \right)_{pH(i)} = f(pH) \quad (2-1)$$

and calculated

$$\frac{RT}{nF} \ln \left(\frac{[M_T]}{[M]} \right)_{pH(i)} = f(pH) \quad (2-2)$$

complex formation curves (ECFC and CCFC, respectively) for the labile on the polarographic time scale metal-ligand systems. In Equations 2-1 and 2-2, $E(M)$, $I(M)$ and $E(comp)$, $I(comp)$ stand for the potential (either half-wave $E_{1/2}$ or peak E_p potential) and current (either limiting diffusion I_d or peak I_p current) in the absence and the presence of metal complexes, respectively.

The total metal ion [M_T] and ligand [L_T] concentrations are known at any stage of the experiment. They can be expressed by the following mass-balance equations

$$[M_T] = [M] + \sum_{p=1} \sum_{q=1} \sum_{r=0} p \beta_{M_p L_q OH_r} [M]^p [L]^q [OH]^r + \sum_{x=1} \sum_{y=1} x \beta_{M_x OH_y} [M]^x [OH]^y \quad (2-3)$$

$$[L_T] = [L] + \sum_{p=1} \sum_{q=1} \sum_{r=0} q \beta_{M_p L_q OH_r} [M]^p [L]^q [OH]^r + [L] \sum_{k=1} \beta_k^H [H]^k \quad (2-4)$$

where $\beta_{M_p L_q OH_r}$ and β_k^H stay for the overall stability and protonation constant, respectively, [M], [L], [H], and [OH] stand for the free metal ion, free ligand, proton and hydroxide concentration, respectively. In case of the formation of complexes involving the protonated form of a ligand, *e.g.* MLH, the term $[M]^p [L]^q [OH]^r$ would be replaced by $[M]^p [L]^q [H]^r$. The refinement of stability constants is achieved by the computation of the free metal ion concentration [M] from mass-balance equations written for the assumed model. The computed [M] is used by Equation 2-2. During the iterative operation, the values of refined stability constants are varied. The refinement operation is completed when the CCFC fits best the experimental objective function, *e.g.* the ECFC.

The potentiometric determination of the protonation constants of TAPSO and stability constants of complexes formed in the system Cu-(TAPSO)_x-(OH)_y made use of the SUPERQUAD [14] and

Equilibrium Simulation for Titration Analysis – ESTA programmes [15] (additional information about ESTA can be found in Part V, Chapter 10, Section 10.2.4.1). During the refinement of the protonation constants the value for pK_w was kept fixed. In the refinement of overall stability constants of copper complexes, the hydrolysis constants of copper (compiled from the literature (Table 2-1)) were introduced as fixed parameters, as well as the protonation constant of TAPSO that was determined in this work.

Table 2-1 Dissociation constant for water, protonation constant for the ligand TAPSO and overall stability constants for Cu^{2+} complexes with OH^- , at 25 °C

	Equilibrium	Log β	I (M)	References
Water	$\text{H}^+ + \text{OH}^- \rightleftharpoons \text{H}_2\text{O}$	13.78	0.1	[16]
TAPSO	$\text{L}^- + \text{H}^+ \rightleftharpoons \text{HL}$	7.55 ± 0.02^a	0.1	This work
Copper	$\text{Cu}^{2+} + \text{OH}^- \rightleftharpoons \text{Cu}(\text{OH})^+$	6.1	0.1	[16]
	$2\text{Cu}^{2+} + \text{OH}^- \rightleftharpoons \text{Cu}_2(\text{OH})^{3+}$	8.2	3.0	[16]
	$\text{Cu}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Cu}(\text{OH})_2$	11.8	0.1	[16]
	$\text{Cu}^{2+} + 3\text{OH}^- \rightleftharpoons \text{Cu}(\text{OH})_3^-$	14.5	1.0	[16]
	$\text{Cu}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Cu}(\text{OH})_4^{2-}$	15.6	1.0	[16]
	$2\text{Cu}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Cu}_2(\text{OH})_2^{2+}$	16.8	0.1	[16]
	$3\text{Cu}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Cu}_3(\text{OH})_4^{2+}$	33.5	0.1	[16]
	$\text{Cu}(\text{OH})_2 (\text{s}) \rightleftharpoons \text{Cu}^{2+} + 2\text{OH}^-$	-18.48	0.7	[16]

^aAverage value and standard deviation calculated from seven independent titrations performed on different solutions.

2.3 RESULTS AND DISCUSSION

2.3.1 PROTONATION CONSTANTS

TAPSO is a zwitterionic buffer, whose structure is shown in Figure 2-1.

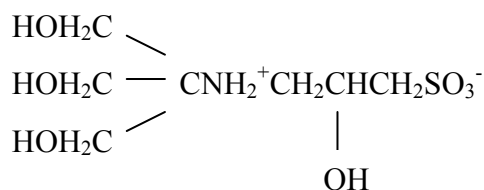


Figure 2-1 Structure of 2-hydroxy-3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid (TAPSO).

The first protonation constant ($\text{p}K_{a1}$) is due to the sulfonic group, the second one is related to a proton on the amino group ($\text{p}K_{a2}$). At a first attempt, we intended to determine both protonation constants of TAPSO. For this purpose, potentiometric data obtained from a set of titrations (about 80 to 150 points for each titration) with standardised KOH (performed at a fixed total TAPSO concentration) were refined using the SUPERQUAD program. It was not possible to determine the exact value of $\text{p}K_{a1}$ but it was verified that at $\text{pH} = 3.0$ the sulfonic group was already deprotonated. The value of the $\text{p}K_{a2}$ was obtained from seven independent titrations on different solutions. The average value and standard deviation in the second protonation constant are reported in Table 2-1. The statistical parameters generated by the program after completion of the refinement operation were within the recommended values: χ^2 was lower than 12, and the standard deviation, σ , was less than 5, at 95% confidence level [14]. The value of $\text{p}K_{a2}$ obtained in this work agrees well with the literature values [7-9, 17].

2.3.2 ADSORPTION STUDIES

Because metal stability constants calculated by polarographic techniques can only be determined in the absence of adsorption of the ligand, ACP at a phase angle of 90° was used to test TAPSO adsorption at the mercury electrode. This phase angle was chosen because a non faradic process is expected and thus mainly the current is capacitive in nature and proportional to the double layer capacity. Figure 2-2 shows a typical example obtained with increasing concentration of TAPSO, at $\text{pH} = 5.55$.

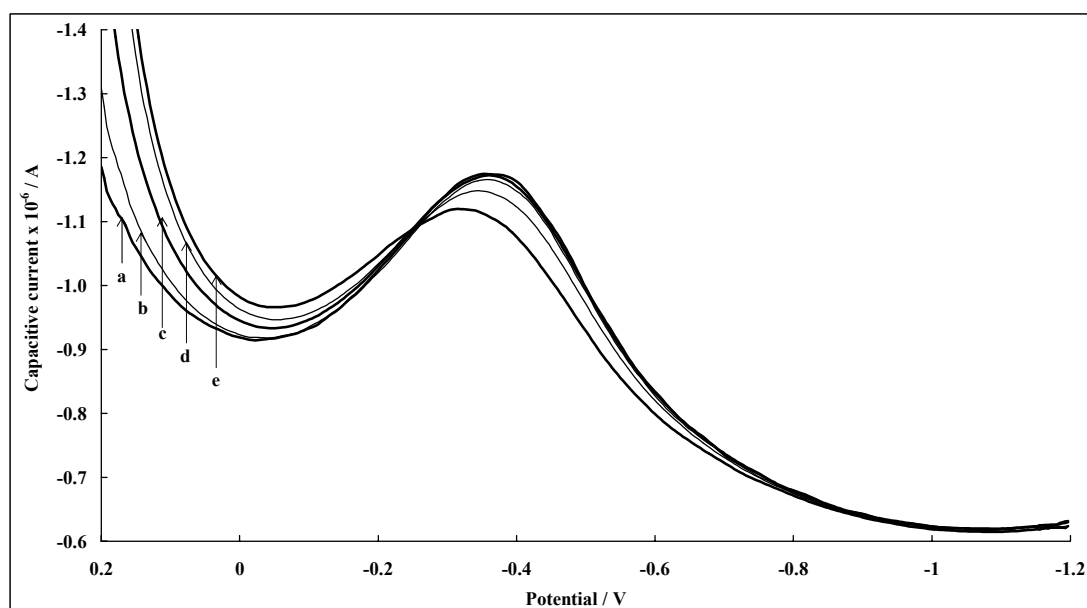


Figure 2-2 AC polarograms for TAPSO solutions in 0.1 M KNO_3 at $\text{pH} 5.55$. $[\text{TAPSO}] =$ (a) 0.0 M; (b) 7.8×10^{-4} M; (c) 1.2×10^{-3} M; (d) 4.8×10^{-3} M; (e) 1.3×10^{-2} M.

For 7.8×10^{-4} M TAPSO, the capacitive current curve (curve b) was superimposed with the curve without pH buffer (0.1 M KNO₃, curve a) in the potential range between 0.05 and -1.2 V showing that no adsorption occurred. For higher pH-buffer concentrations, a slight departure from the curve without pH buffer (0.1 M KNO₃, curve a) was observed in the potential range between 0.2 and -0.7 V (curves c, d and e). These results pointed out a slight adsorption occurrence.

2.3.3 METAL STABILITY CONSTANTS

The analysis of several DCP and DPP titrations, performed at different ligand to metal ratios in a pH range between 3.0 and 10.0, showed that Cu-(TAPSO)_x-(OH)_y system behaved as labile on the polarographic time scale; only one peak corresponding to all labile species present in solution, which shifted to more cathodic potential values, was observed (data not shown).

For both techniques, however, a small departure from fully reversible process was observed. In the case of DPP, the analysis of the width of the peak at half height, $w_{1/2}$, showed values between 62 and 70 mV, *e.g.* some of polarograms had $w_{1/2}$ above 62 mV, the value predicted theoretically [18] for a fully reversible process. Due to this, peak heights were lower than expected. Reversible peak potential for each polarogram was evaluated using procedure reported previously [19]. The equation describing a Gaussian curve was modified to the following form

$$I_r = I_p \exp\{-(E_{appl} - E_p)^2 / [2 \times (w_{1/2} / 2.345)^2]\} \quad (2-5)$$

where I_r stands for the differential reduction current of electrochemically active species recorded at E_{appl} , E_{appl} stands for the applied step potential at which a single measurement of the reduction current is performed, and I_p , E_p and $w_{1/2}$ have a usual meaning in a DPP experiment. The background current I_b was approximated by the use of either a linear equation

$$I_b = aE_{appl} + b \quad (2-6)$$

or a polynomial

$$I_b = aE_{appl}^2 + bE_{appl} + c \quad (2-7)$$

and the overall and recorded differential current was calculated by the addition of I_r and I_b . By fitting experimental curves with fixed peak width set to the expected theoretical value of 62 mV, it was possible to estimate the reversible peak potentials. These computed peak potential values were few mV more anodic than the experimental values. In the case of DCP, the decrease in reversibility did not

affect the limiting current. Equations below were used in fitting the experimental DCP curves.

$$I_F = I_d / \left(10^{\left(\frac{n\gamma(E_{\text{appl}} - E_{1/2})}{0.05916} \right)} + 1 \right) \quad (2-8)$$

$$I_b = a + bE_{\text{appl}} \quad (2-9)$$

$$I_{\text{obs}} = I_F + I_b \quad (2-10)$$

In Equations 2-8 to 2-10, I_F , I_b and I_{obs} stand for the reduction, background and observed total current, n stands for a number of electrons (here 2), E_{appl} is the stepwise applied potential at which the total current was recorded, and γ is a coefficient describing how steep the polarographic wave is; for the fully reversible system γ should be equal to 1. To estimate the value of reversible $E_{1/2}$, I_d and the γ coefficient set to one were kept fixed during the fitting operations. The difference between reversible and observed half wave potential was not larger than few mV. In both cases (DPP and DCP) the corrected values were used in the refinement of the stability constants. Results obtained from DPP (not shown in this work) differed only slightly (a fraction of log unit in stability constants) from those determined by DCP. In this chapter, data from DCP were mainly used for refinement calculations and will be discussed below.

Even though some adsorption of TAPSO was observed at concentrations higher than 1×10^{-3} M, the shapes of the polarographic curves (DCP or DPP) were similar for the various $[\text{L}_T]:[\text{Cu}_T]$ ratios, regardless of the ligand concentrations used in the range 1×10^{-3} and 5×10^{-3} M. Therefore, it was decided to evaluate polarographic data for all $[\text{L}_T]:[\text{Cu}_T]$ ratios.

In Figure 2-3A, a plot of $E_{1/2}$ versus pH, for $[\text{L}_T]:[\text{Cu}_T]$ of 50, is shown. Similar behaviour was observed for the other ratios (data not shown). In this figure, the value of the protonation constant and the respective forms of the ligand are also presented. The prediction of the species and pH-ranges where they are formed and exist as predominant metal containing species can be made from the slopes of straight lines defined in the curve (Figure 2-3A), as it has been recently demonstrated [20]. When only one metal species is reduced in the pH range where one form of a ligand is predominant, a slope of $59.16/n$ mV per pH unit and proton involved in the electrochemical reaction at 25°C (n is the number of the electrons involved in the reaction) should be obtained. According to this, four regions were defined in Figure 2-3A. In the first region, which corresponds to pH value up to $\cong 5.5$, no cathodic shift in $E_{1/2}$ was found. One can easily conclude that in this pH range there was no complexation and the free metal ion is the only species of copper present in solution. In the second region that starts at pH 5.5 a slope of about 30mV per pH unit is observed. This indicates the involvement of one proton in the electrochemical reaction.

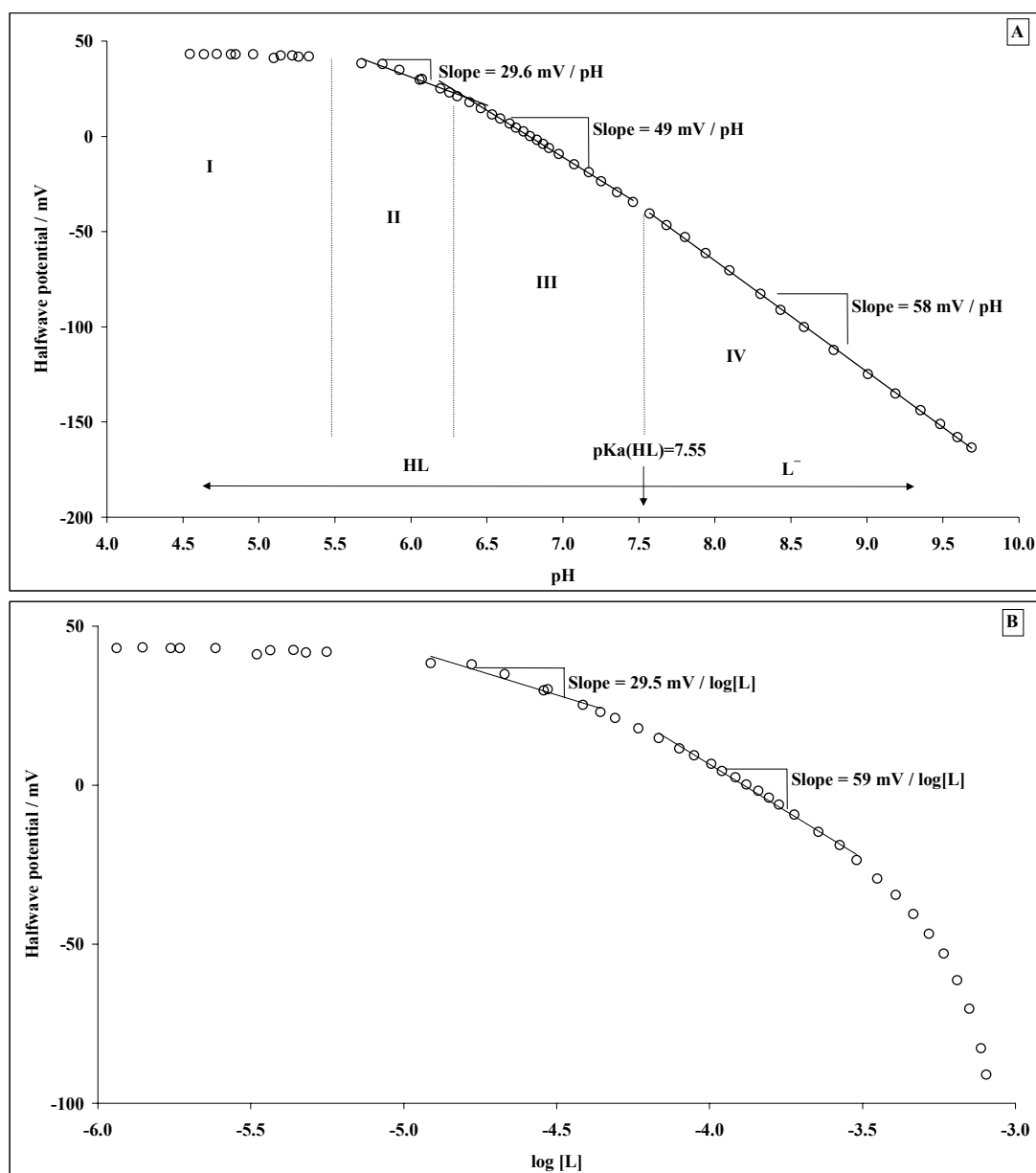
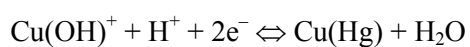
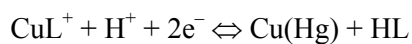


Figure 2-3 Observed shift in the DCP half-wave potential as a function of pH (A) and the free ligand concentration (B) for Cu-(TAPSO)_x-(OH)_y system. [Cu²⁺] = 2.0 × 10⁻⁵ M; [L_T]:[Cu_T] = 50. All polarograms were recorded on the same solution sample titrated with a standard KOH solution.

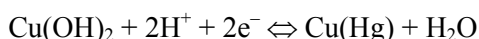
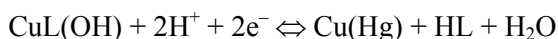
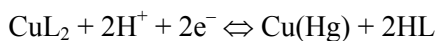
In this pH region one could suggest the reduction of the following complexes at the DME,



Both reactions involve one proton. The reduction of CuL generates HL, which is in agreement with the predominant form of the ligand present in this pH range. Even though, the formation of Cu(OH)⁺

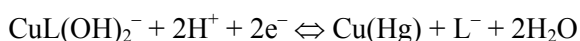
complex meets the criterion of the slope of 30 mV, this species should be excluded from the model as a major component in solution as it is expected to occur at any significant concentration level only at pH higher than 6.5 (see Figure 2-4A).

In region III, a slope of 49mV per pH unit was identified. If two protons were involved in the electrochemical reaction at the DME and one of the following complexes would predominate

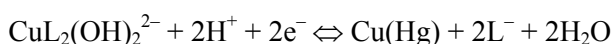


then the expected slope should be 60 mV/pH. However, the experimental slope was smaller (an average between 30 and 60 mV), which means that a mixture of complexes had to be present. One of them must be CuL plus one or several of the metal complexes described just above. In principle, reduction of Cu(OH)₂ should be excluded as a major species since the polarographic signal shifts up to pH 10 and no precipitation was observed, which suggests that the ligand must be involved in the complex formation reaction with copper.

The shift in $E_{1/2}$ continuous to increase to more negative values with the increase in pH. In region IV, a slope of 58mV per pH unit was obtained that corresponds to the involvement of two protons in a electrochemical reaction. In that region, however, the ligand is mainly in the deprotonated form, L⁻. This suggests the formation of hydroxo species with two OH⁻ group. The possible electrochemical reactions, at the DME, are,



and/or



From the above, most likely species to be considered are: CuL⁺, CuL(OH), CuL(OH)₂⁻, CuL₂, and CuL₂(OH)₂²⁻. Due to the combination of species identified, one should also include in the refinement operations CuL₂(OH)⁻ as possible (but not predominant) species to form a logical sequence of complexes: CuL⁺, CuL₂, CuL₂(OH)⁻, and CuL₂(OH)₂²⁻. To confirm the formation of complexes of the type CuL_m, a plot of $E_{1/2}$ versus log [L] for [L_T]:[Cu_T] = 50, was used (see Figure 2-3B). Two different regions can clearly be identified and the slopes confirm the formation of CuL (slope of 29.5 mV) and CuL₂ (slope of 59mV) complexes. Similar variations were observed for other [L_T]:[Cu_T] ratios.

Optimisation of the $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ model and refinement of the stability constants was performed using the experimental (points) and calculated (lines) complex formation curves (Figure 2-4A). Theoretical complex formation curve due to only the formation of $\text{Cu}_x(\text{OH})_y$ complexes was also included in Figure 2-4A (dashed line). As one can see, the difference between the calculated complex formation curve for the system $\text{Cu}-\text{OH}$ (dashed line) and the experimental complex formation curves (empty circles or asterisks) is about two pH units. This indicates the formation of fairly strong copper complexes with the ligand TAPSO. The values of the stability constants of the complexes CuL and CuL_2 included in the initial model (for the refinement operations) were obtained from some preliminary experiments based in DeFord-Hume method [21]. Summation on $\log \beta_{\text{CuL}_n}$ and $\log \beta_{\text{Cu}(\text{OH})_m}$ was used to estimate the initial stability constant values for the $\text{CuL}_n(\text{OH})_m$ species. During the refinement process, it was possible to fit several $\text{Cu}-(\text{L})_x-(\text{OH})_y$ models (see examples in Table 2-2 and Figure 2-4A).

Table 2-2 Overall stability constants for $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ system determined at 25 °C and $I = 0.1$ M by DCP for $[\text{L}_T]:[\text{Cu}_T] = 50$, $[\text{Cu}^{2+}] = 2.0 \times 10^{-5}$ M.

Complex	Equilibrium	Model 1	Model 2a
CuL	$\text{Cu} + \text{L} \Leftrightarrow \text{CuL}$	4.41 ± 0.03	4.42 ± 0.03
CuL(OH)	$\text{Cu} + \text{L} + \text{OH} \Leftrightarrow \text{CuL(OH)}$	11.05 ± 0.36	–
CuL(OH)₂	$\text{Cu} + \text{L} + 2\text{OH} \Leftrightarrow \text{CuL(OH)}_2$	17.99 ± 0.01	–
CuL₂	$\text{Cu} + 2\text{L} \Leftrightarrow \text{CuL}_2$	8.05 ± 0.07	8.08 ± 0.03
CuL₂(OH)	$\text{Cu} + 2\text{L} + \text{OH} \Leftrightarrow \text{CuL}_2(\text{OH})$	–	14.24 ± 0.05
CuL₂(OH)₂	$\text{Cu} + 2\text{L} + 2\text{OH} \Leftrightarrow \text{CuL}_2(\text{OH})_2$	–	20.31 ± 0.01
SD / mV		0.348	0.290

^aIt was possible to replace $\text{CuL}_2(\text{OH})$ by $\text{CuL}(\text{OH})$. SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (see Section 2.3.3).

In all models, CuL and CuL_2 had most consistent stability constants and their presence always resulted in better overall fit whose standard deviation is defined as

$$SD = \sqrt{\frac{\sum (ECFC - CCFC)^2}{N - 1}} \quad (2-11)$$

where N stands for the number of experimental points (curves) recorded on a particular metal-ligand system.

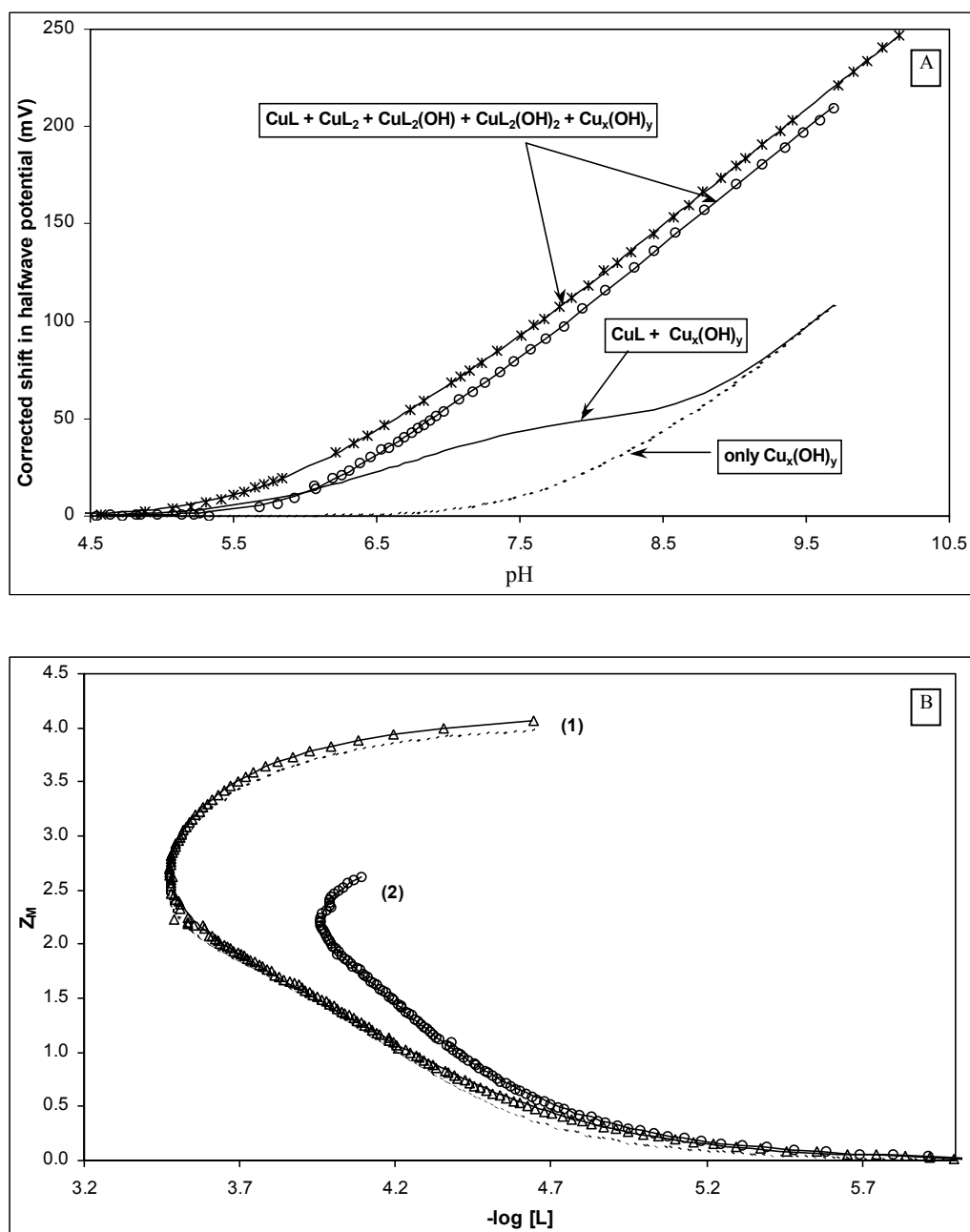


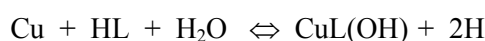
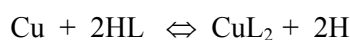
Figure 2-4 Results from refinement and optimisation operations obtained from DCP (A) and GEP (B) experiments for $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ system. A. Experimental (points) and calculated (lines) complex formation curves obtained for the models indicated on the Figure. Circles and asterisks were obtained for $[\text{L}_T]:[\text{Cu}_T] = 50$, $[\text{Cu}^{2+}] = 2.0 \times 10^{-5} \text{ M}$ and $[\text{L}_T]:[\text{Cu}_T] = 100$, $[\text{Cu}^{2+}] = 5.0 \times 10^{-5} \text{ M}$, respectively. Solid lines going through the experimental points were obtained for the optimised models and refined stability constants. B. Observed (points) and calculated (lines) Z_M functions obtained from the refinement operations performed on the GEP data. (1): $[\text{Cu}^{2+}] = 9.442 \times 10^{-4} \text{ M}$, $[\text{L}_T]:[\text{Cu}_T] = 4.04$, dotted line was obtained for the model CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ (Table 2-3, Part B, Model 1); solid line was obtained for the model $\text{CuL}(\text{OH})$ (fixed at 11.45), CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ with simultaneous refinement of $[\text{L}_T]$ that has been adjusted by less than 2 % (Table 2-3, Part B, Model 4). (2): $[\text{Cu}^{2+}] = 9.442 \times 10^{-4} \text{ M}$, $[\text{L}_T]:[\text{Cu}_T] = 3.03$, solid line was obtained for the model CuL , $\text{CuL}(\text{OH})$ and $\text{CuL}(\text{OH})_2$ (Table 2-3, Part A, Model 1).

Fixing CuL at a constant value, to simplify the refinement process, always resulted in CuL₂ with several combinations of CuL_x(OH)_y species (for x and y between 1 and 2) as possible models.

From the above, we concluded that polarographic results were not able to decide on a single and most likely Cu–(TAPSO)_x–(OH)_y model. Since polarographic studies provided data at relatively large [L_T]:[Cu_T] ratios, it was expected that additional experiments using GEP, which works at much lower ratios, might provide significant contribution to the model and stability constants. Potentiometric titrations were carried out as outlined above. The best results were obtained from [L_T]:[Cu_T] ratios of 3 and 4 as no precipitation was observed that was in accord with the prediction made from polarographic results. Two software packages (SUPERQUAD [14] and ESTA [15]) were used in the evaluation of potentiometric data. For each [L_T]:[Cu_T] ratio, the data treatment was done using results from at least two titrations. Stability constants refined by both software programmes were similar.

The refinement operations for each [L_T]:[Cu_T] ratio resulted in different and often acceptable models (see Table 2-3). Different strategies were employed in the refinement operations: (i) refinement of “simple” models without refinement of the analytical parameters, such as initial [H_T] or [L_T] in the cell; (ii) fixing selected stability constants to simplify optimisation procedures; (iii) simultaneous refinement of stability constants with selected analytical parameters. At [L_T]:[Cu_T] ratio of 3, for models containing CuL, CuL₂ and CuL(OH) as well as CuL, CuL₂, CuL(OH) and CuL(OH)₂, the complex CuL₂ was rejected. At the same [L_T]:[Cu_T] ratio, when the model containing CuL, CuL₂, CuL₂(OH) and CuL₂(OH)₂ was investigated then complex CuL₂(OH)₂ was rejected. In the case of [L_T]:[Cu_T] ratio of 4, either CuL(OH) or CuL₂ complex was rejected when initial [L_T] was refined simultaneously with the model containing CuL, CuL₂, CuL(OH) and CuL(OH)₂.

It is important to note that the same amount of protons is involved in the formation of CuL₂ or CuL(OH) (for sake of simplicity the charges were omitted):



which makes it difficult for GEP to distinguish them easily and decide on any of them. Similar situation occurs at higher pH values, where the ligand is fully deprotonated. In this case, neither GEP nor DCP are able to distinguish between CuL₂(OH)₂ or CuL(OH)₂.

Table 2-3 shows that for the [L_T]:[Cu_T] ratio of 4, the best results were obtained with simultaneous optimisation of initial [L_T]; changes in initial [L_T] by less than 2% improved significantly the standard deviations in the computed stability constants and statistical parameters of the overall fit, such as Hamilton R-factor.

Values of the complex formation function, Z_M [22], defined by:

$$Z_M = \frac{[L_T] - L \left(1 + \sum_{n=1}^N \beta_{n01} [H^+]^n \right)}{[M_T]} \quad (2-12)$$

where

$$L = \frac{[H_T] - [H^+] + [OH^-]}{\sum_{n=1}^N \beta_{n01} [H^+]^n} \quad (2-13)$$

were calculated for each datum point (for $[L_T]:[Cu_T]$ ratio of 3 and 4) to aid in the modelling procedures (see Figure 2-4B). The analysis of Z_M functions shows unequivocally that, for both $[L_T]:[Cu_T]$ ratios the complex CuL_2 is formed. The formation of CuL_2 was already evidenced by DCP experiments (see slope of 59 mV per log $[L]$ in Figure 2-3B). This, however, is not in agreement with Model 1, containing CuL , $CuL(OH)$ and $CuL(OH)_2$, for which an excellent refinement results were obtained (Table 2-3, Part A). Z_M function also confirmed that there was no strong evidence to support the formation of species like CuL_3 or CuL_4 . Those species, if present, would have to be at too small concentrations to be detected at the experimental conditions used.

In case of $[L_T]:[Cu_T]$ equal to 4, the experimental Z_M function (triangles in Figure 2-4B) was reproduced fairly well with the model containing CuL , CuL_2 , $CuL_2(OH)$ and $CuL_2(OH)_2$, *e.g.* the Model 1 in Table 2-3, Part B. Since at $[L_T]:[Cu_T]$ of 3 a strong indication of the presence of $CuL(OH)$ was found, any possible attempt was made to incorporate this complex in the metal-ligand model refined for the $[L_T]:[Cu_T]$ ratio of 4. In all cases potentiometric software was always in favour of $CuL_2(OH)$ as well as $CuL_2(OH)_2$, and the same time the complex $CuL(OH)$ was rejected when these three species were allowed to be refined simultaneously. It was possible, however, to obtain excellent statistical outputs from the refinement operations when $CuL(OH)$ was fixed during optimisation procedures that also involved simultaneous refinement of the initial total concentration of the ligand – see solid line for Z_M function 1 in Figure 2-4B and Models 4 and 5 in Table 2-3. For both ratios, a “back-fanning” feature, typical for systems in which complex hydrolysis takes place, was observed (Figure 2-4B).

The analysis of all GEP results showed that this technique was also not able to provide a conclusive answer related to the most likely model; the results from the $[L_T]:[Cu_T]$ ratio of 3 strongly indicated the model CuL , $CuL(OH)$ and $CuL(OH)_2$, whereas those obtained from the $[L_T]:[Cu_T]$ ratio 4 supported the model CuL , CuL_2 , $CuL_2(OH)$ and $CuL_2(OH)_2$. It is important to note that for both ratios, it was possible to refine other species.

From the comparison of all reasonable results obtained by DCP and GEP, the model CuL, CuL(OH), CuL(OH)₂, CuL₂, CuL₂(OH) and CuL₂(OH)₂, for which the overall stability constants, as $\log \beta$, were 4.4 ± 0.1 , 11.8 ± 0.4 , 18.1 ± 0.1 , 8.2 ± 0.2 , 14.6 ± 0.4 and 20.4 ± 0.1 , respectively, could be proposed. Note that standard deviations indicate here the ranges in stability constants obtained from different refined models; when a single model was refined then much smaller standards deviations were obtained for all complexes – see Table 2-3.

Table 2-3 Overall stability constants for Cu–(TAPSO)_x–(OH)_y system determined by GEP, at $[\text{Cu}^{2+}] = 9.44 \times 10^{-4} \text{ M}$, at 25 °C and $I = 0.1 \text{ M}$.

Part A: $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 3$

Complex	Model 1	Model 2	Model 3*	Model 4	Model 5
CuL	4.453 ± 0.002	4.386 ± 0.007	4.467 ± 0.005	4.40 (fixed)	4.40 (fixed)
CuL(OH)	12.111 ± 0.002	-	12.06 ± 0.03	11.71 ± 0.03	11.861 ± 0.007
CuL(OH) ₂	18.160 ± 0.006	-	-	-	-
CuL ₂	-	8.728 ± 0.006	7.22 ± 0.86	8.47 ± 0.03	8.3 (fixed)
CuL ₂ (OH)	-	15.443 ± 0.007	15.08 ± 0.03	15.27 ± 0.02	15.190 ± 0.008
CuL ₂ (OH) ₂	-	-	-	-	-
Hamilton R-factor	0.008	0.018	0.010	0.013	0.014

* CuL₂ and CuL₂(OH) were correlated.

Part B: $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 4$

Complex	Model 1	Model 2	Model 3*	Model 4*	Model 5*
CuL	4.04 ± 0.02	4.1 ± 0.2	4.359 ± 0.006	4.384 ± 0.005	4.416 ± 0.004
CuL(OH)	-	-	-	11.45 (fixed)	11.88 (fixed)
CuL(OH) ₂	-	-	-	-	-
CuL ₂	8.39 ± 0.02	8.33 ± 0.02	8.470 ± 0.005	8.339 ± 0.005	7.99 ± 0.01
CuL ₂ (OH)	14.63 ± 0.04	14.87 ± 0.02	15.00 ± 0.01	14.90 ± 0.01	14.72 ± 0.01
CuL ₂ (OH) ₂	20.49 ± 0.06	-	20.44 ± 0.03	20.52 ± 0.02	20.60 ± 0.01
Hamilton R-factor	0.041	0.045	0.011	0.0096	0.008

* Initial $[\text{L}_\text{T}]$ in the cell was refined simultaneously. Hamilton R-factor stands for a statistical parameter generated by the program ESTA (see Part V, Chapter 10, Section 10.2.4.1).

To test the proposed model, species distribution diagrams (SDD) were generated for $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratios 3 and 4 (GEP experimental conditions) and for the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio 100 (DCP experimental conditions) (Figure 2-5). Figure 2-5A supports the model found at the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio 3, as CuL , $\text{CuL}(\text{OH})$ and $\text{CuL}(\text{OH})_2$ are the major species formed under these experimental conditions. However, Figure 2-5B disagrees with the experimental results obtained at the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio 4, as it suggests that refinement of GEP data obtained at this ratio should also result in CuL , $\text{CuL}(\text{OH})$ and $\text{CuL}(\text{OH})_2$ as most likely model, and not CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$. Figure 2-5C suggests that CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}(\text{OH})_2$ should constitute the most likely model, which is not in agreement with DCP results (Table 2-2).

From the analysis of species distribution diagrams it became clear that all GEP results, *e.g.* refined stability constants for all models considered, might be regarded as too large if one accepts the possibility of formation of all six copper species. It has been noted that with an increase in the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio, an increase in the formation of CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ is observed. Hence it was decided to accept stability constants obtained from DCP for CuL , CuL_2 and $\text{CuL}_2(\text{OH})_2$ as they appeared to be major species formed during this experiment. Stability constants for $\text{CuL}(\text{OH})$, $\text{CuL}(\text{OH})_2$ and $\text{CuL}_2(\text{OH})$ were slightly adjusted in such a way that the generated species distribution diagrams compared well with experimental observations. After this exercise, the final model CuL , $\text{CuL}(\text{OH})$, $\text{CuL}(\text{OH})_2$, CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$, together with the overall stability constants (as $\log \beta$), 4.41, 11.43, 17.55, 8.08, 14.3 and 20.3, respectively, was obtained and used to generate four new SDD (Figure 2-6).

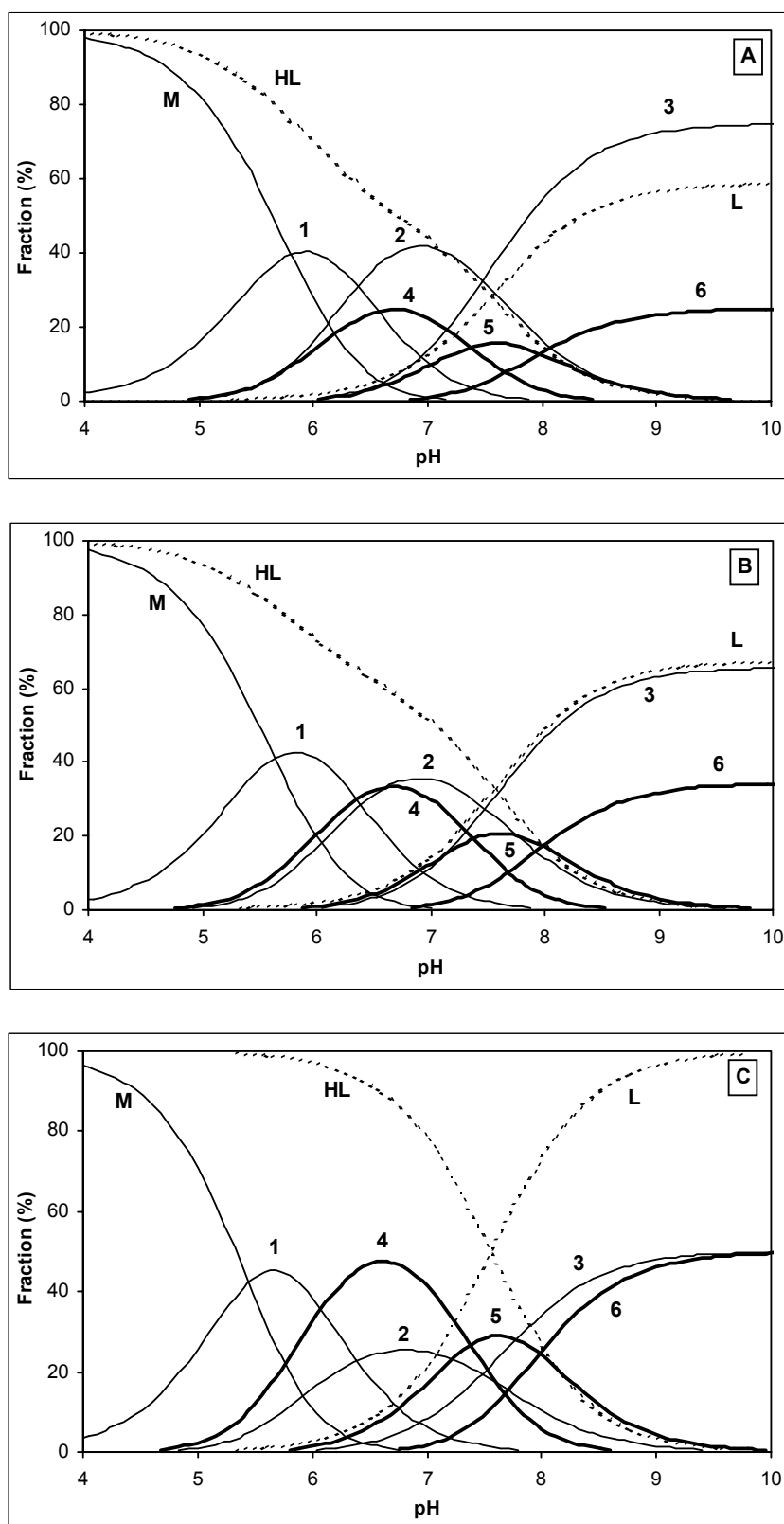


Figure 2-5 Species distribution diagrams computed for Cu-(TAPSO)_x-(OH)_y system: $[L_T]:[Cu_T] = 3$, $[Cu^{2+}] = 9.442 \times 10^{-4}$ M (A), $[L_T]:[Cu_T] = 4$, $[Cu^{2+}] = 9.442 \times 10^{-4}$ M (B), and $[L_T]:[Cu_T] = 100$, $[Cu^{2+}] = 5 \times 10^{-5}$ M (C). The metal-ligand model consisted of CuL (1), CuL(OH) (2), CuL(OH)₂ (3), CuL₂ (4), CuL₂(OH) (5), and CuL₂(OH)₂ (6) for which stability constants, as $\log \beta$, were set to 4.4, 11.8, 18.1, 8.2, 14.6, and 20.4, respectively.

Figure 2-6A predicts, at the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio of 2, mainly the formation of CuL and $\text{CuL}(\text{OH})$ prior the precipitation predicted to occur at about pH 7. GEP experiment, at this $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio, confirmed that it was impossible to collect data in the whole pH-range. Figure 2-6B predicts the absence of precipitation at the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio of 3, which is in agreement with experimental work. This figure also suggests that CuL , $\text{CuL}(\text{OH})$ and $\text{CuL}(\text{OH})_2$ are the major species and for this model the best statistical results were obtained. A significant increase in the concentrations of CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ is predicted in Figure 2-6C for the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio of 4 and these species together with CuL were refined without a problem. Fixing the stability constant for $\text{CuL}(\text{OH})$, it was possible to improve statistical parameters from refinement operations significantly (Model 4 for the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio of 4 in Table 2-3) and this can be easily understood from Figure 2-6C. In addition, CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ are predicted, as major species, at the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio of 100 (Figure 2-6D) (similar situation occurs at a ratio of 50) and this was exactly established from the DCP experiments.

Some recent studies [7-9] have only described the formation of the species CuL and this disagrees with results reported in this work; results from DCP and GEP clearly confirmed the formation of many more complexes. It has been decided to generate the CCFC for this simple model, CuL and $\text{Cu}_x(\text{OH})_y$, as reported in the literature [7-9] (see Figure 2-4A). Only initially, where CuL is formed, there is an overlap between the CCFC generated for this model (CuL) and the results obtained in this work. From pH 6 onwards, the solid line is below the experimental points and confirms that this model is incorrect. The gap between this line and the experimental points strongly supports that more species must be included in the copper-ligand model to fit a CCFC into the ECFC.

In conclusion, the study of metal complexes formed with the monoprotonated ligand TAPSO proved to be a challenge. A single analytical technique, even though if it is as well established as GEP, was not able to provide either reliable model or stability constants. However, the combination of two analytical techniques, GEP and DCP, working at low and high, respectively, $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratios proved to be a powerful tool in the study of metal-ligand equilibria; this combination allowed to generate reliable model and stability constants for five new copper complexes identified with TAPSO.

In this work, polarography with the use of complex formation curves proved to be at least as powerful tool as GEP. Even though adsorption of the ligand on the Hg-working electrode was observed from ACP experiments, it did not influence significantly the model and refined stability constants obtained from DCP.

The observed difference between $\log \beta$ s for $\text{CuL}_2(\text{OH})$ and CuL_2 (about 6.2) is very close to $\log \beta$ for $\text{Cu}(\text{OH})$, *e.g.* 6.1, which suggests that deprotonation of the coordinated water occurs without a significant change in the geometry of the CuL_2 complex. The difference between $\log \beta$ s for $\text{CuL}(\text{OH})$ and CuL (about 7) is significantly larger than $\log \beta$ for $\text{Cu}(\text{OH})$, *e.g.* 6.1. This suggests that the

deprotonation of the coordinated water does change to some extent the geometry of the CuL complex.

Results reported in this work have demonstrated that copper forms stable complexes with TAPSO and as a result the free copper concentration decreases in the presence of 5×10^{-3} M of TAPSO from initial $[\text{Cu}^{2+}] = 5 \times 10^{-5}$ M to 1.3×10^{-6} and 5.0×10^{-10} M at pH 6.5 and 8.5, respectively.

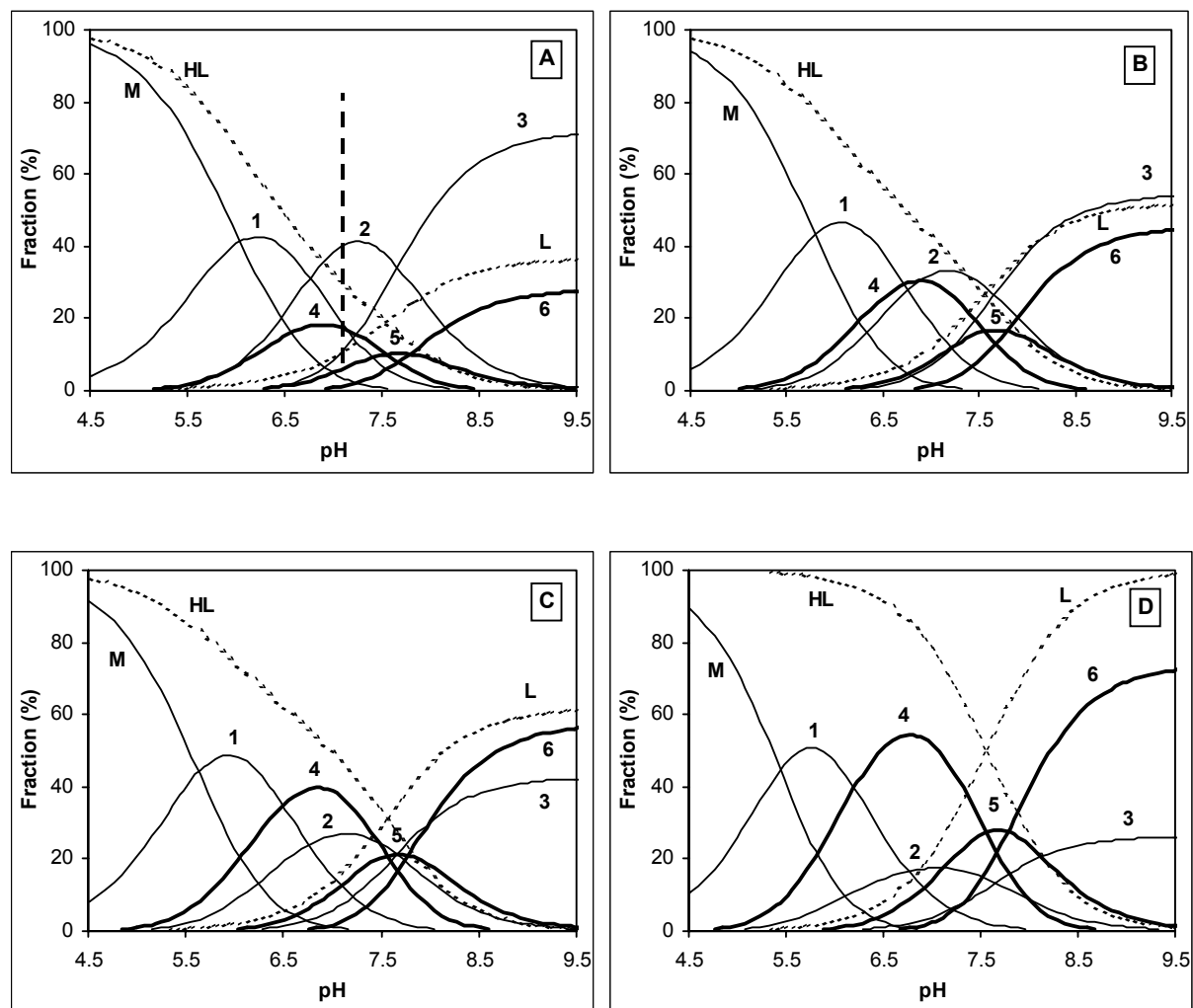


Figure 2-6 Species distribution diagrams computed, for the final model, for Cu-(TAPSO)_x-(OH)_y system. $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 2$, $[\text{Cu}^{2+}] = 9.442 \times 10^{-4}$ M (A), $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 3$, $[\text{Cu}^{2+}] = 9.442 \times 10^{-4}$ M (B), $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 4$, $[\text{Cu}^{2+}] = 9.442 \times 10^{-4}$ M (C), and $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 100$, $[\text{Cu}^{2+}] = 5 \times 10^{-5}$ M (D). The metal-ligand model consisted of CuL (1), CuL(OH) (2), CuL(OH)₂ (3), CuL₂ (4), CuL₂(OH) (5), and CuL₂(OH)₂ (6) for which stability constants, as $\log \beta$, were set to 4.41, 11.43, 17.55, 8.08, 14.3, and 20.3, respectively.

3.2.2.2 POLAROGRAPHY

Polarographic measurements were performed with a computer controlled instrumental set-up [27] and a Model 663 VA stand (Metrohm) equipped with a multimode electrode (Metrohm, model 6.1246.020) as a working electrode, used in the dropping mercury electrode mode. A silver/silver chloride (3M KCl) electrode and a platinum electrode (both Metrohm) were used as reference and counter electrodes, respectively. The pH of the solutions was measured to $\pm 0.1\text{mV}$ (± 0.001 pH units) with a PHI 72 pH meter (Beckman) using a Model 6.0733.100 a silver/silver chloride, 3 M KCl reference and a Model 6.0133.100 glass electrodes, both from Metrohm.

3.2.3 PROCEDURE

3.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

The measurements of $\text{pH} = -\log [\text{H}^+]$ (for all techniques described above) were performed after the calibration of the glass electrode. This calibration was achieved by a potentiometric titration of nitric acid with potassium hydroxide (both standardised solutions). The values in E° and response slope were obtained by fitting a straight line in the experimental points collected around pH 2 and 11.

3.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

ACP polarograms were run in the absence of the metal ion to evaluate the adsorption of the ligand on the surface of the mercury working electrode. The experimental conditions used were: amplitude of sinusoidal alternating current of 5mV, frequency of 75 Hz, scan rate of 2.5 mV/s and a phase angle at 90° . The polarograms were scanned in the potential range between 0.2 and -1.2 V at three different pH values (8.0, 9.0 and 10.0), varying the ligand concentrations from 4.0×10^{-4} to 1.3×10^{-2} M. These pH values were chosen because they correspond to experimental conditions where AMPSO occurs as HL (100%), HL and L^- (50% of each) and L^- (100%), respectively. In this way the adsorption of all forms of the ligand could be examined.

3.2.3.3 GLASS ELECTRODE POTENTIOMETRY

Seven potentiometric titrations in the pH-range between 3.2 to 11.0 (using a monotonic or dynamic titrant additions) were performed to establish the model and determine the stability constants for the $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system. For each titration, between 70 and 200 points were collected.

3 CHALLENGES IN MODELLING AND OPTIMISATION OF STABILITY CONSTANTS IN THE STUDY OF METAL COMPLEXES WITH DIPROTONATED LIGANDS. A GLASS ELECTRODE POTENTIOMETRIC AND POLAROGRAPHIC STUDY OF Cu-(AMPSO)_x-(OH)_y SYSTEM²

ABSTRACT

The influence AMPSO on systems containing copper(II) was studied by GEP, and DCP, at fixed total ligand to total metal concentration ratios and various pH values (25° C, 0.1 M KNO₃ medium). The predicted model (CuL⁺, CuL(OH), CuL₂, CuL₂(OH)⁻, CuL₂(OH)₂²⁻ and CuL₃⁻), and the overall stability constants for species found were obtained by combining results from both electrochemical techniques. The last five complexes are reported for the first time. For the species CuL⁺, CuL₂, CuL₃⁻, and CuL₂(OH)₂²⁻ it was possible to determine stability constants with reasonable certainty and their values, as log β, were found to be 4.62 ± 0.04, 9.5 ± 0.1, 13.4 ± 0.1, and 21.2 ± 0.1, respectively. For the species CuL(OH) and CuL₂(OH)⁻, stability constants 11.7 ± 0.2 and 15.6 ± 0.2, respectively, are presented as indicative values. It was demonstrated that AMPSO buffer may decrease the free copper concentration by ten orders of magnitude by forming complexes with copper. For the first time, the correction in DCP waves for the adsorption of the ligand and quasi-reversibility of the metal allowed to determine stability constants values, which are in good agreement with the values obtained by GEP. The importance of graphic analysis of data and significance of employing two analytical techniques was demonstrated; neither GEP nor DCP would be able to provide the correct Cu-(AMPSO)_x-(OH)_y model and reliable stability constants when used independently.

3.1 INTRODUCTION

In the last three decades, Good and co-workers have synthesized several hydrogen ion buffers for biological research [1, 3]. These buffers were designed taking into account several criteria, among them (i) they should have maximum water solubility and minimum solubility in other solvents to keep them from accumulating in the biological phase and preventing of passing through the membranes, (ii) there should be a minimal effect of temperature and ionic strength on their pK_a values, (iii) the buffers should be stable and must not absorb light above 240nm (iv) they should be easy to prepare and inexpensive (v) they should not form complexes with cations, or if they do, the complexes should be soluble and their stability constants should be known [1, 3].

² Helvetica Chimica Acta 86 (2003) 3288-3304

In fact, some zwitterionic N-substituted aminosulfonic acids do not complex cations, as it was already demonstrated previously [23-26]. However, there are others buffers, such as TAPSO that forms several complexes with copper (Part II, Chapter 2). AMPSO has a structure similar to that of TAPSO, and one might also expect that strong copper complexes of this buffer would be formed when AMPSO is used to control the pH. Both compounds, TAPSO and AMPSO, are commercially available (*e.g.* from Sigma-Aldrich) as biological buffers. However, besides their pK_a values no information is provided on their complexing abilities with *e.g.* biologically important metal ions.

In this work, we report the overall stability constants for species formed in the $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system at 25° C and ionic strength 0.1 M KNO_3 . Three electrochemical techniques, DCP, DPP and GEP, were employed. Five new complexes of copper with AMPSO were identified. Experiments by ACP were also carried out to check buffer adsorption at the mercury working electrode surface, under the experimental conditions used. A simple correction in DCP waves (for the adsorption of the ligand and quasi-reversibility of the electrochemical reduction of the metal ion) was successfully applied in the determination of stability constants.

3.2 EXPERIMENTAL

3.2.1 MATERIALS AND REAGENTS

The ligand AMPSO (99.1%) was obtained from Sigma-Aldrich (St Louis, Missouri, USA) and used as received.

Additional information about the remaining reagents and experimental conditions was described previously (Part II, Chapter 2, Section 2.2.1).

3.2.2 APPARATUS

3.2.2.1 POTENTIOMETRY

The potentiometric experiments reported in this work were performed as previously described (Part II, Chapter 2, Section 2.2.2.2).

The Cu^{2+} concentration was kept at about 1.0×10^{-3} M and a $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio of 9.1 was obtained by addition of appropriate amount of the ligand AMPSO.

3.2.3.4 DIRECT CURRENT AND DIFFERENTIAL PULSE POLAROGRAPHY

Two polarographic techniques (DC and DP) were simultaneously performed on the same solution sample to evaluate the electrochemical behaviour of the system $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$. A drop time of 2 s and a step potential of 4 mV were used for both techniques. In the DPP measurements, a pulse height of 50 mV was used, with pulse width and integration time of 200 ms and 60 ms, respectively. A typical polarographic experiment consisted of the steps described previously in Part II, Chapter 2, Section 2.2.3.3. Several different total ligand to total metal ion concentration ratios ($[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$) were used. For $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio equal to 155, 271, and 72 the total copper concentrations were 4.9×10^{-5} , 7.5×10^{-6} , and 1.5×10^{-5} M, respectively. A set of between 40–60 polarograms (representing all the species present at each pH value) was recorded, between 2.8 and 10, for each titration. The equilibrium of the metal-ligand solutions was tested and it was reached in a few minutes.

3.2.4 DATA TREATMENT

AMPSO, a zwitterionic buffer, has two protonation constants; the first is due to the sulfonic group and the other involves the amino group. In a previous work (Part II, Chapter 2), we determined the $\text{pK}_{\text{a}2}$ for a related zwitterionic buffer, TAPSO. In addition, it has been verified that the sulfonic group of TAPSO was already deprotonated below pH of 3. The value of $\text{pK}_{\text{a}2}$ of TAPSO established previously was in very good agreement with the values reported in the literature [7-9, 17]. Therefore, in the case of AMPSO, we decided to use the literature $\text{pK}_{\text{a}2}$ value of 9.05 [28] determined for the experimental conditions as employed in this work (Table 3-1).

The program ESTA [15] (for more details about the program see Part II, Chapter 2, Section 2.3.3 and Part V, Chapter 10, Section 10.2.4.1) was used to analyze the potentiometric data.

The refinement of polarographic data was done using the Cukrowski method [12, 13]; as it was described previously (Part II, Chapter 2, Section 2.2.4).

During the refinement operations the K_{w} [16], protonation constant for the ligand AMPSO [28] as well as the formation constants for the copper hydroxide species [16] were kept at fixed values seen in Table 2-1 and Table 3-1.

Table 3-1 Protonation constant for the ligand AMPSO, at 25 °C.

Equilibrium	Log β	I (M)	Reference
$L^- + H^+ \rightleftharpoons HL$	9.05	0.1	[28]

3.3 RESULTS AND DISCUSSION

3.3.1 LIGAND ADSORPTION AND COPPER REVERSIBILITY STUDIES

ACP at a phase angle of 90° was used to test whether there was adsorption of AMPSO at the mercury electrode. The studies showed that there was a small but significant ligand activity (in the range of potential where copper is reduced) particularly at $[L_T]$ larger than 1.2×10^{-3} M (data not shown).

Two polarographic experiments, with $[L_T]:[Cu_T]$ ratios of 271 and 155, involved the ligand concentration larger than 1.2×10^{-3} M. Analysis of DC and DP polarograms obtained for these two $[L_T]:[Cu_T]$ ratios did not show a significant increase or decrease in the recorded current, but the shape of recorded waves and peaks varied significantly from expected curves. For instance, for the $[L_T]:[Cu_T]$ ratio of 155, the DPP peak width ($w_{1/2}$) and the gamma coefficient for DCP waves varied between 62 and 65.5 mV and between 0.9 and 0.6, respectively. These values indicated that the behaviour of the system, besides the influence of adsorption, could be regarded as not fully reversible [18]. The refinement of polarographic data [12, 13] employed in this work requires that the recorded curves to represent fully reversible electrochemical process if accuracy in obtained stability constants is of interest. As far as we know, there is yet neither theory nor a suitable mathematical data treatment that would allow a direct use of experimental results obtained from quasi reversible electrochemical processes. It is known that the limiting diffusion current I_d does not depend on the degree of reversibility. Thus, we decided to use a simple procedure for correcting the departure from the reversibility of the DCP data, which is illustrated in Figure 3-1. The DCP wave is a sigmoid curve and can be reproduced by a set of equations already described in Part II, Chapter 2 (Equations 2-8 to 2-10). Theoretically, if the process is fully reversible, then the γ coefficient should be equal to one. By fixing this coefficient at one and keeping sufficient number of experimental points (circles) it was possible to obtain the corrected limiting diffusion current, I_d , and half-wave potential, $E_{1/2}$, from the computed curves, as it is illustrated in Figure 3-1.

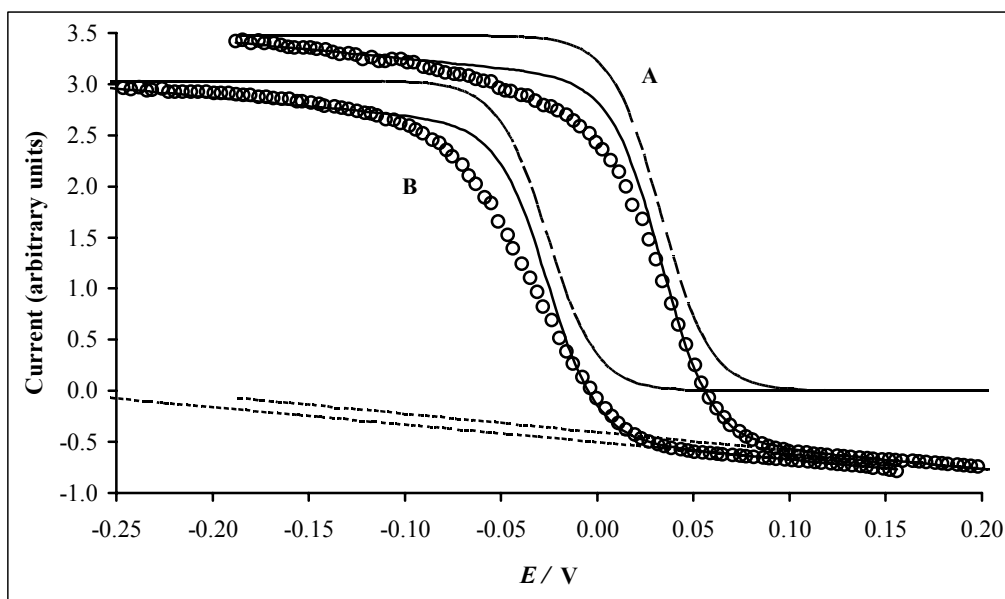


Figure 3-1 Illustration of the fitting procedure of the DC polarograms for $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system. A: $[\text{Cu}^{2+}] = 4.9 \times 10^{-5} \text{ M}$ in background solution, 0.1 M KNO_3 ; B: solution A after the addition of $7.5 \times 10^{-3} \text{ M}$ of the ligand AMPSO, adjusted to $\text{pH} = 6.60$ by addition of KOH. Circles – experimental points; dotted lines – computed background current, Equation 2-9, solid lines – computed DC curves for fully reversible process, Equation 2-10, (I_d and $E_{1/2}$ was used from those fitted curves in the computation of stability constants); dashed lines – computed reduction current for fully reversible process, Equation 2-8.

The number of experimental points allowed in the fitting operation was varied in such a way that the theoretically computed DC wave reproduced the background current (dotted lines in Figure 3-1) and the current at the foot of the wave well, and the limiting current was parallel to the background current when going through experimental points obtained at significantly more negative potential when compared with $E_{1/2}$. The above procedure was used to compute corrected I_d and $E_{1/2}$ for all DC polarograms. Attempts to correct DPP experimental curves, as described by Equations 2-5 to 2-7 in Part II, Chapter 2 were not successful. This shows that DCP technique is by far more suitable technique to use in cases of $\text{M}-(\text{L})_x-(\text{OH})_y$ systems that are not fully reversible.

3.3.2 MODELLING PROCEDURES AND REFINEMENT OF STABILITY CONSTANTS

Prior to any refinement operation, a reasonable $\text{Cu}-(\text{L})_x-(\text{OH})_y$ model and suitable guesses in the stability constants for the proposed complexes must be prepared. Evaluation of the GEP data was performed using ESTA program [15]. Values of the complex formation function Z_M , defined previously (Part II, Chapter 2, Section 2.3.3), were calculated for each datum point. The analysis of the

Z_M function, represented in Figure 3-2 for one titration and a selected $\text{Cu}-(\text{L})_x-(\text{OH})_y$ model as an example, is a helpful tool to understand the system as it allows to predict the formation of some complex species just by graphic analysis.

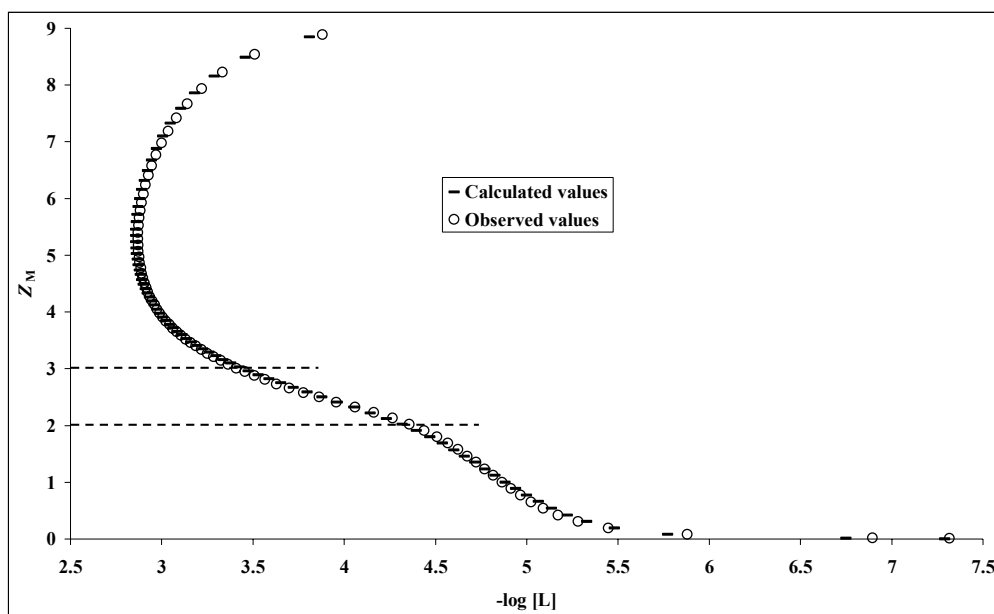
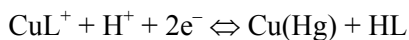


Figure 3-2 Z_M function for $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system calculated from the refinement operations performed on the GEP data by ESTA program. The computed Z_M function was obtained for CuL^+ , CuL_2 , CuL_3^- and $\text{CuL}_2(\text{OH})_2^{2-}$ (Model 1 in Table 3-2). $[\text{L}_T]:[\text{Cu}_T] = 9.1$; $[\text{Cu}^{2+}] = 8.97 \times 10^{-4}$ M, at 25 °C and 0.1 M KNO_3 .

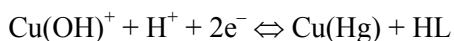
The analysis of Z_M function clearly indicates the formation of CuL_2 and CuL_3^- as the major metal containing species because the Z_M function shows typical and stepwise sigmoid increase to the value of 3. Above the value of 3, the Z_M function shows a typical feature (back-fanning) for the formation of major species containing OH groups. The formation of CuL^+ complex as a major species is not seen in Figure 3-2; the Z_M function continuously increases from 0 to 2 without showing a plateau at value of 1. If the complex CuL^+ is formed then its formation is immediately followed by the formation of CuL_2 (CuL^+ must be then a minor metal containing species). It is important to stress that the Z_M function can only be generated for the assumed $\text{Cu}-(\text{L})_x-(\text{OH})_y$ model. This implies, that one has to investigate numerous possible models and generate Z_M functions for all of them in order to select most probable one, usually based on the observed fit of the theoretical function with the objective (experimental) one.

The analysis of several DCP titrations, performed at different ligand to metal ratios in a pH range between 2.8 and 10, showed only one DCP wave. This wave shifted towards more cathodic potential and represents all labile metal containing species $\text{Cu}_p(\text{AMPSO})_x(\text{OH})_y$ present in the solution. The direct graphic analysis of the experimental DCP data (Figure 3-3) corroborated the information

obtained from GEP data. From the analysis of the shift in $E_{1/2}$ vs. pH (Figure 3-3A) it is seen that there is no slope of 30mV per pH unit; this slope is characteristic for a two-electron reduction process at the DME involving one proton, *i.e.*

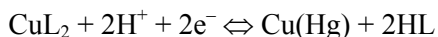


or/and

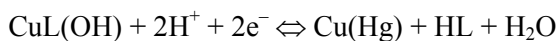


This supports the conclusion arrived at from the analysis of the GEP results that if the complex CuL^+ is formed then it is only to a small extent. It is important to mention that the formation of Cu(OH)^+ must not be rejected from the overall $\text{Cu-(L)}_x\text{-(OH)}_y$ model as this is the pH-range where its formation is, indeed, expected.

A slope of 59 mV per pH is observed throughout more than one pH unit – see the points in the pH-range between 6.5 and 7.5 in Figure 3-3A. This clearly indicates the formation of CuL_2 or CuL(OH) species according to electrochemical reactions involving two protons, *i.e.*



or/and



In principle, one must also consider the formation of Cu(OH)_2 , the reduction of which also would involve two protons, *i.e.*

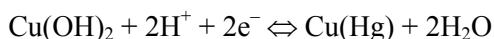
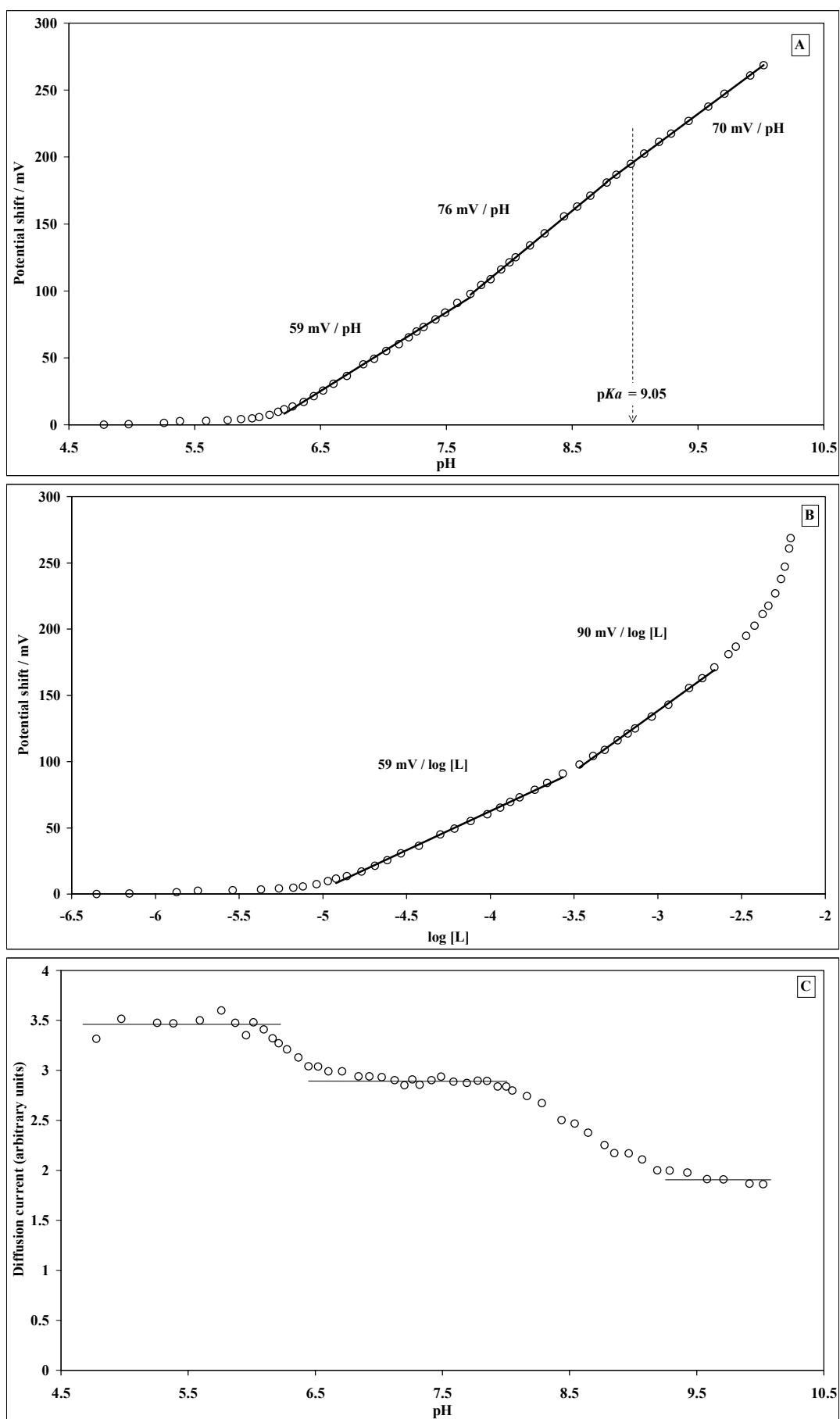
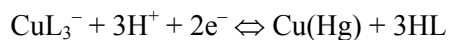


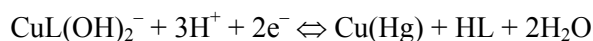
Figure 3-3 Analysis of the DCP data obtained from the $\text{Cu-(AMPSO)}_x\text{-(OH)}_y$ system at 25°C and 0.1 M $\rightarrow$ KNO_3 ionic strength. (A) – Shift in the corrected half-wave potential as function of pH; (B) – Shift in the corrected half-wave potential *versus* the computed free ligand concentration; (C) Variation in diffusion current (obtained after correction shown in Figure 3-1) *versus* pH. $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 155$, $[\text{Cu}^{2+}] = 4.9 \times 10^{-5}$ M.



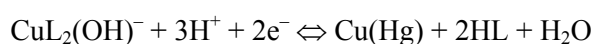
In the pH range between 7.5 and 9.0, an increase in the slope from 59 to 77 mV per pH unit was observed, suggesting the formation of CuL_3^- , $\text{CuL}(\text{OH})_2^-$ or $\text{CuL}_2(\text{OH})^-$ according to the following electrochemical reactions involving three protons, *i.e.*



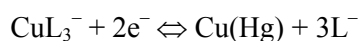
or/and



or/and

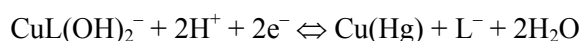


The theoretically expected slope when three protons are involved is about 90 mV. The experimental slope did not reach the theoretical value of 90 mV/pH because the protonation constant of the ligand AMPSO is about 9 and electrochemical processes without an involvement of protons must also take place, *e.g.*

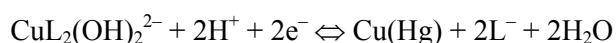


hence, the observed slope is a resultant slope caused by numerous electrochemical reactions where, on average, the number of protons involved is between two and three. Note that, in the electrochemical reaction at the DME involving three protons, one also has to consider the formation of $\text{Cu}(\text{OH})_3$.

Above pH 9, the ligand is mainly in the deprotonated form L^- . The observed decrease in the slope from 77 (in the pH range below $\text{p}K_{\text{a}2}$) to 70 mV per pH unit (above $\text{p}K_{\text{a}2}$) fully confirms the formation of species $\text{CuL}_x(\text{OH})_y$. If one assumes that $\text{Cu}_x(\text{OH})_y$ were solely present throughout the experiment, then a continuous increase in the slope would be observed with an increase in the pH. This increase in the slope would be due to the consecutive increase in the number of OH groups involved in the complex formation. In addition, precipitation of $\text{Cu}(\text{OH})_2(\text{s})$ would be expected, and that was not observed. In other words, the decrease in the slope at $\text{pH} \approx \text{p}K_{\text{a}2}$ could only be explained if the ligand was involved in the complex formation reaction with the metal ion throughout the whole pH-range. The observed slope of 70 mV confirmed the formation of $\text{CuL}_x(\text{OH})_2$ species, such as



or/and



It is important to realize that, in the pH range above the $\text{p}K_{a2}$ value (where mainly L^- exists), the experimental slope depends only on the number of OH groups involved in the complex formation reaction. This means that one is unable to establish the number of L involved in the complex formation reaction from relationship seen in Figure 3-3A. The theoretically expected slope of 60 mV might mean the formation of $\text{CuL}(\text{OH})_2$, $\text{CuL}_2(\text{OH})_2$, ... $\text{CuL}_x(\text{OH})_2$, all of which contain two OH groups.

From the above, it is obvious that additional information is required to suggest most likely model, particularly at high pH values. The analysis of the graph $E_{1/2}$ vs. $\log [\text{L}]$ (Figure 3-3B) is used to confirm the formation of Cu_pL_q species [12]. Once again, there was no evidence of CuL^+ formation (no slope of 30 mV was identified). The well-defined slopes of 59 and 90 mV confirmed unequivocally the formation of CuL_2 and CuL_3^- , respectively, as major species present in solution. Now it has become clear that the large shift, if not the largest shift reported to date from the polarographic studies of metal complexes, observed in Figure 3-3A, cannot be attributed only to the formation of $\text{Cu}_x(\text{OH})_y$. As a matter of fact, all the above indicates that the species $\text{Cu}_x(\text{OH})_y$ are not formed to a large extent in the pH-range investigated in this study.

In Figure 3-3C, the I_d determined after the correction shown in Figure 3-1, was plotted against the pH of the solution. Three regions can be distinguished: (i) up to pH of about 6, where I_d remains at the level recorded for the metal ion solution, indicating that significant amount of complexes with the ligand AMPSO was not formed; (ii) a well-defined pH region between 6.4 and 7.8, where I_d had very much same value indicating the formation of a major species in the solution (perhaps CuL_2 as CuL was excluded as a major solution component), (iii) above pH of about 7.8 a decrease in I_d was observed indicating the formation of consecutive species like CuL_3^- , $\text{CuL}_x(\text{OH})_2^{x-}$, etc. The recorded current seems to asymptotically approach a constant value above pH 9.5 suggesting the presence of another predominant species there, possibly $\text{CuL}_2(\text{OH})_2$, if the above discussion and analyses of slopes seen in Figure 3-3A, are taken into consideration.

From the above, it was possible to propose most likely $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ model that included CuL^+ (possibly), CuL_2 , CuL_3^- , $\text{CuL}_x(\text{OH})_2^{x-}$, where x most likely stands for 1 and 2. To establish a sequence in the formation of complexes, one also should include $\text{CuL}_x(\text{OH})^{(1-x)}$ in the overall initial model that consisted of CuL^+ , CuL_2 , CuL_3^- , $\text{CuL}(\text{OH})$, $\text{CuL}(\text{OH})_2^-$, $\text{CuL}_2(\text{OH})^-$ and $\text{CuL}_2(\text{OH})_2^{2-}$ plus all known $\text{Cu}_x(\text{OH})_y^{2x-y}$ species.

The next step was to refine the GEP data with the program ESTA. It immediately appeared that ESTA was able to refine several different models with refined stability constants in a wide range for the same species. The overall statistical parameter, R-factor, did not provide enough information on rejection or acceptance of a particular model. It became obvious that a special strategy had to be developed in order to propose a final $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ model. The strategy, as well as the results

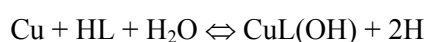
obtained, are illustrated in Table 3-2. Note that all refinement operations were carried out without the simultaneous optimization of analytical parameters, such as $[H^+]$, $[L^-]$, or E° . First, we started by including in the $Cu-(AMPSO)_x-(OH)_y$ model all the seven $CuL_x(OH)_y$ complexes mentioned above. ESTA always rejected $CuL(OH)_2^-$ and $CuL_2(OH)$, regardless of the initial values in the stability constants. After removing these two complexes from the model, the program ESTA was randomly rejecting either $CuL(OH)$ or CuL_2 , converging to either Model 1 (CuL^+ , CuL_2 , CuL_3^- and $CuL_2(OH)_2^{2-}$), or Model 2 (CuL^+ , CuL_3^- , $CuL(OH)$ and $CuL_2(OH)_2^{2-}$) – see Table 3-2.

Table 3-2 Overall stability constants for $Cu-(AMPSO)_x-(OH)_y$ system refined with ESTA program from GEP data; $[L_T]:[Cu_T] = 9.1$; $[Cu^{2+}] = 8.97 \times 10^{-4}$ M, 25 °C and 0.1 M KNO_3 . Simultaneous refinement of data collected from four independent potentiometric titrations (762 points).

Complexes	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
CuL^+	4.60 ± 0.02	4.65 ± 0.02	4.61 ± 0.02	4.62 ± 0.02	4.63 ± 0.02	4.60 ± 0.02
CuL_2	9.69 ± 0.02	-	9.60 (fixed)	9.52 ± 0.02	9.28 ± 0.04	9.54 ± 0.02
CuL_3^-	13.54 ± 0.02	13.51 ± 0.02	13.54 ± 0.02	13.53 ± 0.02	13.52 ± 0.02	13.3 (fixed)
$CuL(OH)$	-	12.23 ± 0.02	11.53 ± 0.07	11.7 (fixed)	12.0 (fixed)	11.7 (fixed)
$CuL(OH)_2^-$	-	-	-	-	-	-
$CuL_2(OH)^-$	-	-	-	-	-	15.52 ± 0.05
$CuL_2(OH)_2^{2-}$	21.33 ± 0.02	21.35 ± 0.02	21.34 ± 0.02	21.33 ± 0.02	21.34 ± 0.02	21.32 ± 0.02
Hamilton R-factor	0.0145	0.0143	0.0145	0.0144	0.0143	0.0146

Hamilton R-factor stands for a statistical parameter generated by the program ESTA (see Part V, Chapter 10, Section 10.2.4.1)

This strongly indicated that ESTA was not able to distinguish between CuL_2 and $CuLOH$. One must realise that the formation of these two complexes occurs in the pH-range where HL is the major form of the ligand (Figure 3-4), and it involves the same amount of protons (for sake of simplicity, the charges of the complexes will be omitted)



The statistical data treatment (refinement) involving CuL_2 illustrates difficulties in making final decisions based only on mathematical operations, *e.g.* based only on the R-factor; one could easily

propose a model without the complex CuL₂. However, the rejection of CuL₂ is not supported by the graphic analysis of Z_M function (Figure 3-2) and by DCP data (slope of 59 mV seen in Figure 3-3B). The complex CuL₂ is unequivocally present in solution. After fixing one of these complexes (CuL(OH) or CuL₂), the program ESTA was able to refine the other stability constant (Models 3 to 5 in Table 3-2).

The program ESTA always accepted CuL₃ and CuL₂(OH)₂, regardless of the presence of other complexes included in the model. The refined stability constants for these two complexes, as log β, were 13.53 ± 0.04 and 21.35 ± 0.02, respectively (note that the standard deviations indicate here the ranges in stability constant values obtained from different refined models). The refinement operations performed on potentiometric data suggest that CuL₃ and CuL₂(OH)₂ are most stable copper complexes formed with the ligand AMPSO. This can be used as a strong support of the supposition that these two complexes, CuL₃ and CuL₂(OH)₂, are octahedral and the ligand AMPSO acts here as a bidentate ligand with N-atom and one alcoholic O-atom as donor atoms involved in the bonds with copper.

It was possible to refine stability constants for the complex CuL₂(OH) but only when other complexes were kept fixed (see Model 6 in Table 3-2). As one can see in Figure 3-2, with the model CuL, CuL₂, CuL₃ and CuL₂(OH)₂ an almost perfect fit was obtained between the calculated and the experimental values. A similarly good fit, however, was obtained when *e.g.* CuL(OH) and CuL₂(OH) were included in the model – see Model 6 in Table 3-2. In this case, the analysis of the Z_M function generated for different models was of no help in deciding on most likely and final model Cu-(AMPSO)_x-(OH)_y model because the observed differences were not significant enough – very much the same Hamilton R-factor was obtained for all models seen in Table 3-2.

The refinement of the stability constant from DCP data was performed by means of the ECFC and CCFC. Some of the refinement results are shown in Table 3-3. The results confirmed some observations and conclusions made from refinement of the GEP data. The complexes CuL₂, CuL₃ and CuL₂(OH)₂ appeared to be most ‘favorite’ species throughout all the refinement operation; they had to be the major species in the solution (Model 1, Table 3-3) as predicted from the analysis of the experimental data seen in Figure 3-3. The values of the stability constants obtained for CuL₃ and CuL₂(OH)₂, calculated from DCP experiments (Model 1, Table 3-3), were in good agreement with those refined from potentiometric titrations (Models 1 and 2, Table 3-2). These results show that, except for CuL₂ (for more details see below), the correction performed for adsorption and the departure from reversibility was efficient. Moreover, CuL(OH)₂ does not seem to form at any significant concentration since it was rejected by DCP and ESTA refinement operations; as a matter of fact, GEP and DCP showed that complex CuL(OH)₂ appeared to be the least likely species formed in a solution.

The analysis of the stability constants obtained by both electrochemical techniques, compiled in Table 3-2 and Table 3-3, suggests some observations. Regarding the $\log K_{\text{CuL}}$, ESTA refined this value to 4.62 ± 0.04 (note that the standard deviation indicates here the ranges in stability constant values obtained from different refined models). The DCP program was not able to give an estimate for this stability constant, the main reasons being (i) the large excess of CuL_2 , (ii) possibly not enough data points collected prior the formation of CuL_2 , or (iii) that the contribution brought by the experimental points related to the formation of CuL was not significant enough for the refinement operations. However, fixing $\log K_{\text{CuL}}$ at the GEP or slightly lower value resulted in better statistical refinement of the polarographic data. In addition, the computed complex formation curve for different values in the stability constant for CuL confirmed that it could not be larger than 4.7, otherwise the calculated formation curve would never fit the experimental one. From ESTA refinement, $\log \beta_{\text{CuL}_2}$ varied for different models between 9.2 and 9.7. In DCP refinement, the value was larger, around 10. If the value of the stability constant for CuL was 4.6, then the expected $\log \beta_{\text{CuL}_2}$ value should be about 9.2 (twice the $\log K_{\text{CuL}}$). This significant difference between GEP and DCP results for CuL_2 might be due to some influence of adsorption of the ligand on DCP results in this pH range. In addition, it is possible that the slightly larger GEP value in $\log \beta_{\text{CuL}_2}$ in the Model 1 seen in Table 3-2 was due to the fact that GEP was unable to refine simultaneously CuL_2 and CuL(OH) stability constants.

Table 3-3 Overall stability constants for $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system obtained from DCP data, 25 °C and 0.1 M KNO_3 , $[\text{L}_\text{T}]:[\text{Cu}^\text{T}] = 155$, $[\text{Cu}^{2+}] = 4.9 \times 10^{-5}$ M.

Complexes	Model 1	Model 2
CuL^+	4.62 (fixed)	4.62 (fixed)
CuL_2	9.95 ± 0.02	9.95 ± 0.02
CuL_3^-	13.51 ± 0.02	13.3 ± 0.3
CuL(OH)	-	-
CuL(OH)_2^-	-	-
$\text{CuL}_2(\text{OH})^-$	-	15.7 ± 0.4
$\text{CuL}_2(\text{OH})_2^{2-}$	21.19 ± 0.01	21.16 ± 0.02
SD / mV	1.755	1.725

SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (see Part II, Chapter 2, Section 2.3.3).

If one assumes that CuL(OH) is formed to some extent, then the value for $\log \beta_{\text{CuL}_2}$ would be expected to be lower. From the analysis of all the data, it appears that CuL(OH) species might be formed but in a smaller amount when compared with CuL_2 . In addition, we verified the formation of CuL(OH) in a

similar system, Cu-(TAPSO)_x-(OH)_y (Part II, Chapter 2). The inclusion of CuL(OH) complex in the model brings the stability constant value of CuL₂ down to a more reasonable value (9.4 ± 0.2) (Models 3 to 6, Table 3-2).

If CuL(OH) is formed, its stability constant value of about 11.5-12.0 (Models 3 to 6, Table 3-2) is much larger than the expected value from the theoretical point of view, $\log K_{\text{CuL}} + \log K_{\text{CuOH}} = 4.6 + 6.1 = 10.7$. It is difficult to justify this value. A similar behavior was described with the system Cu-(TAPSO)_x-(OH)_y system, for which stability constant of CuL(OH) (as $\log \beta$) was found to be 11.43, much larger than the theoretical value, 10.51 (Part II, Chapter 2). Maybe, the ‘excessively’ large stability constants for CuL₂ and CuL(OH) might be explained by a change in the geometry of the complexes.

CuL(OH)₂ does not seem to be formed at all at any significant concentration since it was rejected by ESTA and DCP refinements.

In general, CuL₂(OH) was rejected by ESTA, except for Model 6 (Table 3-2) where CuL₃ was fixed. However, CuL₂(OH) was readily accepted by DCP refinement (Model 2, Table 3-3) with an overall fit very similar to that obtained for Model 1 in Table 3-3. The average value from DCP and GEP, $\log \beta_{\text{CuL}_2(\text{OH})}$ of about 15.6 ± 1 , is well in the range of the theoretically predicted value ($\log \beta_{\text{CuL}_2} + \log \beta_{\text{Cu(OH)}} = 9.2$ (or 9.6) + $6.1 = 15.3$ to 15.7). The large standard deviations associated with the refined stability constants for CuL₃ and CuL₂(OH) (Model 2, Table 3-3) were due to the simultaneous refinement of both complexes. It was enough to fix one of them to generate a reasonable standard deviation for the other complex. If CuL₂(OH) is formed, it must be a transitional species in a sense that almost immediately the second OH group is coordinated to form a very stable complex, CuL₂(OH)₂ with possibly octahedral configuration. The computed value for this complex is exactly in the expected range ($\log \beta_{\text{CuL}_2} + \log \beta_{\text{Cu(OH)}_2} = 9.2$ (or 9.6) + $11.8 = 21.0$ to 21.3), and it suggests that the formation of the CuL₂(OH)₂ complex does not involve a significant change in the geometry of complexes involved. This might suggest that the complex CuL₂ is also octahedral with water or counter ion molecules in axial positions, and this is why the stability constant of this complex is somewhat larger than expected when compared with the value established for the complex CuL. If that supposition holds, then the complex CuL₂(OH) must also be octahedral with possibly OH and water (or counter ion) in axial positions. The complex CuL₂(OH)₂ is a major and ‘favorite’ species accepted by both refinement operations (potentiometric and polarographic) with computed stability constant of about 21.2 ± 0.1 (Table 3-2 and Table 3-3).

In GEP refinement, ESTA always refined CuL₃ with a $\log \beta$ very constant (13.53 ± 0.04) indicating that this complex is a predominant species in the solution. A similar value for the stability constant of CuL₃ was obtained from polarography except the situation when CuL₃ and CuL₂(OH) were

refined simultaneously. If complex $\text{CuL}_2(\text{OH})$ is formed, then it coexists with CuL_3 at the same pH (Figure 3-4E and Figure 3-4F), and the decrease in the computed stability constant of CuL_3 is expected. If one assumes that $\text{CuL}_2(\text{OH})$ is formed to some extent, then the range of the value of $\log \beta_{\text{CuL}_3}$ could be 13.3 ± 0.3 , when combining the results obtained from both techniques. If a stability constant value of, *e.g.*, 13.0 is assumed, then the species distribution diagram computed for the experimental conditions used for DCP ($[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 155$, $[\text{Cu}^{2+}] = 4.86 \times 10^{-5} \text{ M}$) shows that $\text{CuL}_2(\text{OH})$ is a major species in the pH-range where CuL_3 coexists (data not shown). However, this observation is not in agreement with the experimental slope of 90 mV/log [L] seen in Figure 3-3B, as well as with the variation in the Z_M function seen in Figure 3-2. Next, the stability constant for CuL_3 was slightly adjusted in such a way that the generated calculated complex formation curves, for which different values of CuL_3 were introduced in the presence of fixed values of CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$, compared well with the experimental complex formation curve (data not shown). After this exercise, we concluded that the value for $\log \beta_{\text{CuL}_3}$ should be 13.4 ± 0.1 and this is well within the range of 13.3 ± 0.3 indicated above.

To test the proposed models, SDD were generated for the $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratios 9.1 and 155 (the GEP and DCP experimental conditions, respectively) - Figure 3-4. Figure 3-4A and Figure 3-4B, corresponding to the model CuL , $\text{CuL}(\text{OH})$, CuL_3 and $\text{CuL}_2(\text{OH})_2$, are compatible with Figure 3-3A, but clearly disagree with the experimental results shown in Figure 3-2 and Figure 3-3B where a plateau around 2 for Z_M function and a slope of 59 mV/log [L], respectively are well defined. On the other hand, Figure 3-4C and Figure 3-4D, suggest that CuL , CuL_2 , CuL_3 and $\text{CuL}_2(\text{OH})_2$ should constitute the most likely model, as it correlates very well with the experimental results presented in Figure 3-2 and Figure 3-3. However, if we assume that both CuL_2 and $\text{CuL}(\text{OH})$ are formed, as well as $\text{CuL}_2(\text{OH})$ (Model 6, Table 3-2), then two new SDD can be generated (Figure 3-4E and Figure 3-4F). These figures predict, at $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratios 9.1 and 155, mainly the formation of CuL_2 , $\text{CuL}_2(\text{OH})_2$, and CuL_3 , which is in perfect agreement with the main conclusions obtained for the models refined in Table 3-2 and Table 3-3. Figure 3-4F, suggests a small increase of CuL , without a significant concomitant increase of CuL_2 in the pH range between 5 and 6.3, which is in agreement with a slight cathodic shift of the potential (Figure 3-3A). Even though $\text{CuL}(\text{OH})$ and $\text{CuL}_2(\text{OH})$ are present (as minor species), Figure 3-4F, confirms that CuL_2 and CuL_3 are the predominant species in the pH range where typical slopes of these species were identified (Figure 3-3B).

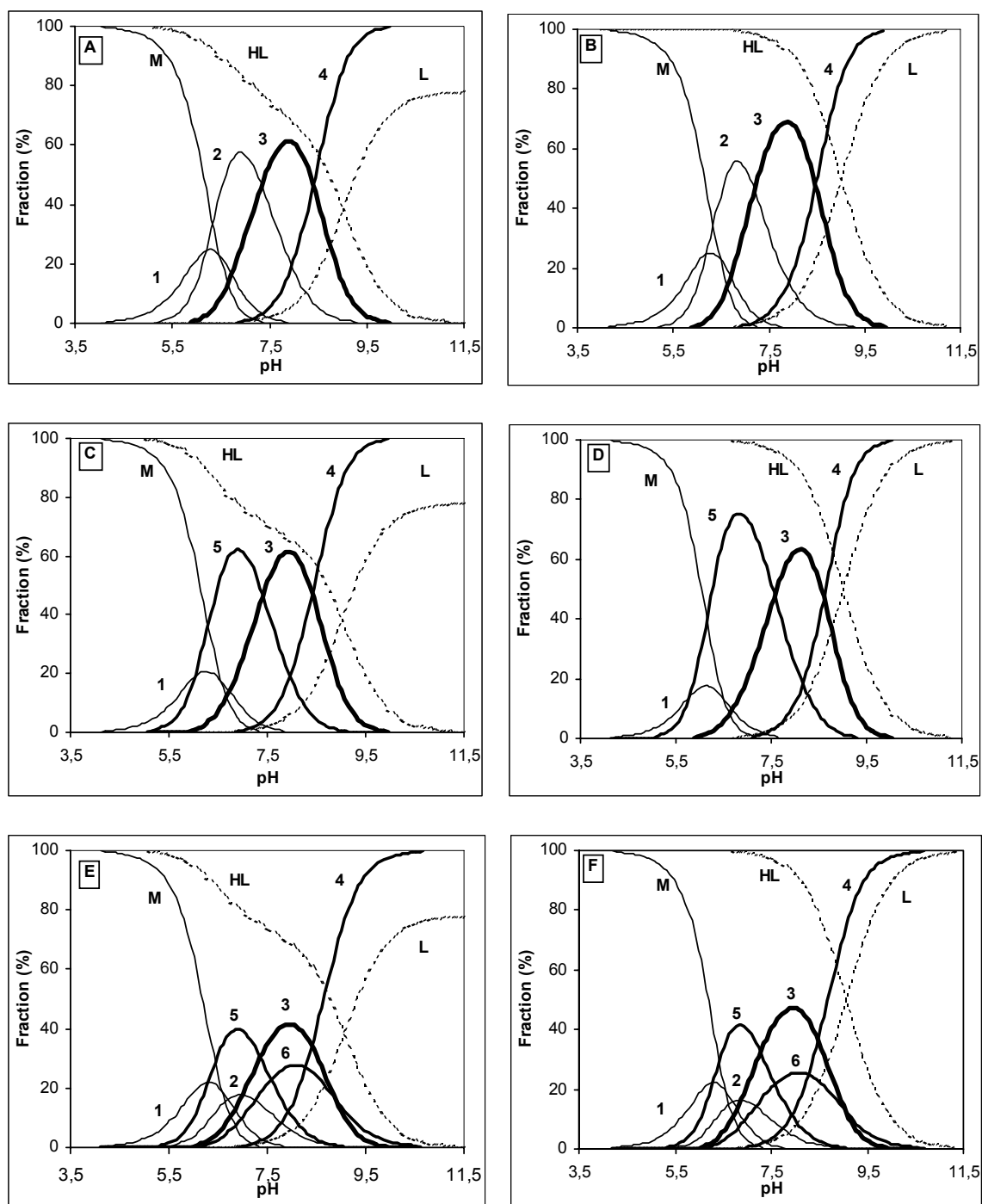


Figure 3-4 Species distribution diagrams computed for different $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ models. A, C and E – GEP experimental conditions, $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 9.1$, $[\text{Cu}^{2+}] = 8.97 \times 10^{-4}$ M; B, D and F – DCP experimental conditions, $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 155$, $[\text{Cu}^{2+}] = 4.9 \times 10^{-5}$ M. For all $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ models, the overall stability constants, as $\log \beta$, are shown: A and B – CuL (1), $\text{CuL}(\text{OH})$ (2), CuL_3 (3) and $\text{CuL}_2(\text{OH})_2$ (4), with stability constants set to 4.65, 12.23, 13.51 and 21.35, respectively; C and D – CuL (1), CuL_2 (5), CuL_3 (3) and $\text{CuL}_2(\text{OH})_2$ (4), with stability constant set to 4.6, 9.69, 13.51 and 21.33 (C), or 4.62, 9.95, 13.51 and 21.19 (D), respectively; E and F – CuL (1), $\text{CuL}(\text{OH})$ (2), CuL_2 (5), $\text{CuL}_2(\text{OH})$ (6), CuL_3 (3) and $\text{CuL}_2(\text{OH})_2$ (4), with stability constants set to 4.62, 11.7, 9.5, 13.4, 15.7 and 21.2, respectively. For sake of simplicity, charges were omitted in the figure.

From the comparison of all reasonable results obtained by GEP and DCP, the model CuL , CuL(OH) , CuL_2 , $\text{CuL}_2(\text{OH})$, $\text{CuL}_2(\text{OH})_2$ and CuL_3 could be proposed. However, from the considerations above, it is clear that it is impossible to provide stability constants for all the species with high certainty. The most certain values were obtained for CuL , CuL_2 , CuL_3 and $\text{CuL}_2(\text{OH})_2$ and they are, as $\log \beta$, 4.62 ± 0.04 , 9.5 ± 0.1 , 13.4 ± 0.1 and 21.2 ± 0.1 , respectively. Note that the standard deviations indicate here the ranges in stability constant obtained from different models refined by both techniques. For CuL_2 and CuL_3 , the values were obtained after some iterative processes described above. For CuL(OH) and $\text{CuL}_2(\text{OH})$ complexes, only indicative stability constant can be proposed, which are 11.7 ± 0.2 and 15.6 ± 0.2 , respectively. It is also important to mention that CuL , CuL(OH) and $\text{CuL}_2(\text{OH})$ are minor and CuL_2 , CuL_3 and $\text{CuL}_2(\text{OH})_2$ are major species formed under the GEP and DCP conditions (Figure 3-4E and Figure 3-4F). In Table 3-4, the final model with the overall stability constants for $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system determined by GEP and DCP is presented.

Table 3-4 Overall stability constants for copper complexes with AMPSO obtained in this work, after combining polarographic and potentiometric results, at 25 °C and 0.1 M ionic strength.

Equilibrium	Log β
$\text{Cu}^{2+} + \text{L}^- \rightleftharpoons \text{CuL}^+$	4.62 ± 0.04
$\text{Cu}^{2+} + 2\text{L}^- \rightleftharpoons \text{CuL}_2$	9.5 ± 0.1
$\text{Cu}^{2+} + 3\text{L}^- \rightleftharpoons \text{CuL}_3^-$	13.4 ± 0.1
$\text{Cu}^{2+} + \text{L}^- + \text{OH}^- \rightleftharpoons \text{CuL(OH)}$	11.7 ± 0.2
$\text{Cu}^{2+} + 2\text{L}^- + \text{OH}^- \rightleftharpoons \text{CuL}_2(\text{OH})^-$	15.6 ± 0.2
$\text{Cu}^{2+} + 2\text{L}^- + 2\text{OH}^- \rightleftharpoons \text{CuL}_2(\text{OH})_2^{2-}$	21.2 ± 0.1

In a previous work (Part II, Chapter 2), we reported that TAPSO forms six main species with copper: CuL , CuL(OH) , CuL(OH)_2 , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$. The comparison of the models for TAPSO and AMPSO points out, as a major difference, that TAPSO was not able to form CuL_3 . The logical explanation is probably related to the crowded atom arrangement around the N-atom of TAPSO, which does not allow this ligand to form more than CuL_2 .

Figure 3-4F shows the SDD for the final model proposed, CuL , CuL(OH) , CuL_2 , $\text{CuL}_2(\text{OH})$, $\text{CuL}_2(\text{OH})_2$ and CuL_3 , for DCP experimental conditions used. This SDD shows that AMPSO binds copper strongly, and as a result, the free copper concentration decreases in the presence of 7.5×10^{-3} M AMPSO from initial $[\text{Cu}^{2+}] = 4.9 \times 10^{-5}$ M to 3.2×10^{-9} and 2.4×10^{-14} M at pH 8.0 and 10.0, respectively. Almost ten orders of magnitude decrease in the free copper concentration in the pH-range where AMPSO is efficient as a buffer can be regarded as a huge change. These results should warn the

users of Good buffers that AMPSO must not be used for studies involving copper ions in solution unless the extent of complexation is accounted for. Taking into account all of what was stated above, it is clear that AMPSO forms several strong complexes with copper. Our results disagree entirely with the literature model described by other authors [28] in which only the formation of CuL was reported.

Stability constants of kinetically labile complexes of amalgam-forming metal ions can be calculated from the dependencies of their DC polarograms. However, it has been shown that when the ligand is specifically adsorbed at the electrode surface, the calculated stability constants differ significantly from the constants obtained by potentiometric measurements [29, 30]. It has been proved in this work that, after a simple correction performed on the experimental data, the values of the stability constants determined by DCP, even with a slight adsorption of the ligand at the mercury electrode and a quasi-reversible behavior of the metal ion, are in good agreement with the values obtained by GEP.

In conclusion, this $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system was a real challenge to model and determine the stability constants. Once again, it was proved that it is very important to use more than one technique to confirm the results; this is even more important when the system is very complicated. As a matter of fact, analytical techniques, when employed individually, were not able to provide the final model and reliable stability constants. The polarographic technique of speciation and refinement of stability constants employed in this work proved to be as powerful as well-established procedures employed in GEP. It was also shown that a graphical analysis was very helpful and important not only in the prediction of the initial model, but also for verifying of the stability constants obtained for different models considered.

4 CHALLENGES IN MODELLING AND OPTIMISATION OF STABILITY CONSTANTS IN THE STUDY OF Cu-(TAPS)_x-(OH)_y SYSTEM BY POLAROGRAPHIC³

ABSTRACT

This work describes the application of polarography, a technique scarcely used for modelling and optimisation of stability constants, in the study of copper complexes with TAPS. Due to the simple structure of the ligand and its high basicity, no other available techniques for determining complex equilibrium constants would be able to characterize properly the Cu-(TAPS)_x-(OH)_y system in the buffering pH range. DCP, using low total copper ion and large total ligand to total copper concentration, enabled the full characterization of Cu-(TAPS)_x-(OH)_y system, whose complexation occurs in the pH range of copper hydrolysis and Cu(OH)₂ precipitation. Cu-(TAPS)_x-(OH)_y system was studied by DCP and GEP in aqueous solution at fixed total ligand to total metal concentrations ratios and varied pH values (25.0 °C; *I* = 0.1 M, KNO₃). The predicted model, as well as the overall stability constants values, are (as log β): CuL = 4.2, CuL₂ = 7.8, CuL₂(OH) = 13.9 and CuL₂(OH)₂ = 18.94. GEP only allowed confirming the stability constants for CuL and CuL₂ and was used to determine the p*K*_{a2} of TAPS, 8.342. Finally, a briefly comparative analysis between TAPS and other structural related buffers was done. Evaluation based on log β_{CuL} versus p*K*_{a2} revealed that TES, TRIS, TAPS and AMPSO coordinated via amino and hydroxymethylgroups forming a five-membered chelate ring. For BIS-TRIS and TAPSO, and possibly DIPSO, one or more five-membered chelate rings involving additional hydroxyl groups are also likely formed.

4.1 INTRODUCTION

Hydrogen buffers are used to maintain a constant concentration of hydrogen ions in solution. The hydrogen donor/acceptor can be oxygen, as is the case of phosphate; however, for many biological buffers nitrogen acts as a donor/acceptor since a variety of substituents can tailor the amine p*K*_a to neutral pH. Nowadays, several chemical suppliers devote a special section of their catalogues, where a long list of popular amine-based buffers is available [31, 32].

Usually researchers obtain buffer conditions from previous publications. The primary concern is that buffers do not participate in the reaction system through inhibition, activation or complexation. It

³ To be submitted for publication

is usually assumed that initial investigations for buffer controls have been performed and that reported results are free of buffer artefacts. However, in most of the cases, these studies were not performed and several interferences are described in the literature where metal ions complexation by these biological buffers occurs. To exemplify this statement, two cases are presented: (i) the enzymatic activity of acetylcholinesterase (AChE) is inhibited when TRIS is used to prepare the Ellman's reaction solution [33] and (ii) the generation of fluorescence, browning and polymerization of lysozyme, when using the TAPSO buffer, were markedly inhibited under both aerobic and non-aerobic conditions [34]; these interferences are probably due to their (TRIS or TAPSO) metal complexation.

Common buffers used in various analytical, biochemical and other applications involving metal ions [34-36] include zwitterionic buffers such as AMPSO, BIS-TRIS, DIPSO, TAPSO, TES or TRIS. These buffer anions possess a primary (TRIS), secondary (AMPSO, TAPSO or TES) or a tertiary (DIPSO and BIS-TRIS) amine function, and also β alcohol groups (structures were included in Table 4-3), which can all potentially coordinate to metals. In fact, it has been shown that all of these buffers complex with copper ions in solutions especially at their buffering pH range ([16, 37], Part II, Chapters 2 and 3). TAPS is also a zwitterionic buffer widely used in various applications [38-41]. This buffer is structural related with the biological buffers described above. Thus, it is expected that TAPS also complexes copper and therefore it is fundamental the knowledge of the stability constants values for taken into account when complexation of the buffer with copper ion takes place.

In the present work, we report the overall stability constants for the species formed between TAPS and Cu^{2+} , at 0.1 M KNO_3 ionic strength and $25.0 \pm 0.1^\circ\text{C}$. The characterization of the system was done with the employment of two electrochemical techniques, DCP and GEP. Experiments by ACP were also carried out to check if this buffer adsorbs at the mercury working electrode surface, under the experimental conditions used. TAPS' protonation constant was also determined by GEP.

4.2 EXPERIMENTAL

4.2.1 MATERIALS AND REAGENTS

The ligand TAPS (99.5 %) was purchased from Sigma-Aldrich (St Louis, Missouri, USA) and was used as received. The copper standard solution, 3.1×10^{-2} M, was purchased from Merck.

Additional information about the remaining reagents and experimental conditions was described previously (Part II, Chapter 2, Section 2.2.1).

4.2.2 APPARATUS

All experiments were carried out in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer, and thermostatted at 25.0 ± 0.1 °C using a water bath.

4.2.2.1 POLAROGRAPHY

Polarographic measurements were performed using a Model 663 VA stand (Metrohm) equipped with a multimode electrode (Metrohm, Model 6.1246.020), as a working electrode, used in the dropping mercury electrode mode. A silver/silver chloride (3M KCl) and a glassy carbon were used as reference and counter electrodes (both from Metrohm), respectively. The VA stand was attached to a microAutolab (Eco Chemie, Utrecht, The Netherlands) system controlled by a personal computer. The pH measurements were conducted with a GLP 22 pH meter Crison (Barcelona, Spain) with a sensitivity of $\pm 0.1\text{mV}$ (± 0.001 pH units) with a silver/silver chloride reference electrode (Metrohm) and a glass electrode (Metrohm).

4.2.2.2 POTENTIOMETRY

The potentiometric experiments reported in this work were performed as previously described (Part II, Chapter 2, Section 2.2.2.2).

4.2.3 PROCEDURE

4.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

For all techniques used, the calibration of the glass electrode (pH measured as $-\log [\text{H}^+]$) was accomplished as previously described (Part II, Chapter 3, Section 3.2.3.1).

4.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

Adsorption studies of TAPS were performed by ACP. Experiments were run, in the absence of copper, for buffer concentrations ranging from 8.0×10^{-4} to 1.4×10^{-2} M at three different pH values: 6.34, 8.34 and 10.34. These pH values were chosen because they correspond to experimental conditions where TAPS occurs as HL (100%), HL and L^- (50% of each) and L^- (100%), respectively,

and thus, allows to analyse the adsorption effects in all forms of the ligand. The experimental conditions used were: an amplitude of sinusoidal alternating current of 5 mV, a frequency of 75 Hz and a scan rate of 2.5mV/s; the capacitive current (i_{ac}) was measured at a phase angle of 90°. The potential range scanned was from 0.2 to -1.2 V *versus* Ag/AgCl(s), 3 M KCl.

4.2.3.3 GLASS ELECTRODE POTENTIOMETRY

Firstly, GEP was used to determine the protonation constants of the buffer at $25.0 \pm 0.1^\circ\text{C}$ and 0.1M KNO_3 ionic strength. Several titrations were performed using a total of three different TAPS solutions containing 6.0×10^{-3} or 1.0×10^{-2} M in 0.1M KNO_3 . For each titration, about 300 points were collected between pH 4.9 and 11.9. For $\text{Cu}-(\text{TAPS})_x-(\text{OH})_y$ system, four titrations (two independent solutions) were carried out, between pH 3.4 and 7.0 (for each titration, about 40 points were collected), with a total ligand to total copper concentration ($[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$) ratio set to 10.0, $[\text{Cu}_\text{T}] = 1.0 \times 10^{-3}$ M. For both cases, monotonic additions of standardized KOH 0.1M were done.

4.2.3.4 DIRECT CURRENT POLAROGRAPHY

The study of the electrochemical behaviour of $\text{Cu}-(\text{TAPS})_x-(\text{OH})_y$ system was performed by DCP. A step potential of 4 mV and a drop time of 1 and 2s (for the lower copper concentration), were used. Two $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratios were run. The total copper concentration was 7.0×10^{-6} M for 151.6 $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio and 4.0×10^{-5} M for 97.5 $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratio. Polarographic titrations were performed between pH 3.9 and 10.5; in this way, for each titration, about 50 polarograms were obtained. The procedure used to collect the data points was identical to the one described previously (Part II, Chapter 2, Section 2.2.3.3). The equilibrium of the metal-ligand solutions was tested and it was reached in a few minutes.

4.2.4 DATA TREATMENT

The modelling and optimization procedures of polarographic data were done using the Cukrowski method [12, 13]; as it was described previously (Part II, Chapter 2, Section 2.2.4).

The simulation and optimization of the models and complex formation constants from potentiometric titrations data were performed using ESTA program [15, 42]. For more details about the program, see Part II, Chapter 2, Section 2.3.3 and Part V, Chapter 10, Section 10.2.4.1.

The protonation constant for water [16], copper hydroxide formation constants, $\text{Cu}_x(\text{OH})_y$ [16]

(Table 2-1) and protonation constant for TAPS (Table 4-1) were kept fixed during the refinement operations.

Table 4-1 Protonation constant for the ligand TAPS.

Equilibrium	Log β	I (M)	Reference
$\text{L}^- + \text{H}^+ \rightleftharpoons \text{HL}$	8.342 ± 0.001	0.1	This work

4.3 RESULTS AND DISCUSSION

4.3.1 CALCULATION OF TAPS PROTONATION CONSTANTS

TAPS is a zwitterionic buffer, which has two acidic sites, the sulfonic ($\text{p}K_{a1}$) and the amino ($\text{p}K_{a2}$) groups (see insert in Figure 4-1). The first protonation constant is released from the sulfonic group at a rather low pH ($\text{p}K_{a1}$ value less than 3.0, see Section 2.3.1), which is not relevant for the present study since it was not expected that TAPS complexes copper at pH values lower than 5.0. Potentiometric data were refined using ESTA program and the refinement of $\text{p}K_{a2}$ performed, between pH 7.4 and 9.4 using 483 points, gave a value of 8.342 ± 0.001 with a Hamilton R-factor (see Part V, Chapter 10, Section 10.2.4.1) of 0.0153.

4.3.2 LIGAND ADSORPTION AND REVERSIBILITY STUDIES

ACP at a phase angle of 90° was used to test if there was adsorption of TAPS at the mercury electrode surface. Figure 4-1 shows a typical example obtained with increasing TAPS concentration at pH = 10.34 and 0.1M KNO_3 . Comparing the AC polarograms recorded with the background solution (0.1 M KNO_3 , curve a, Figure 4-1) with the polarograms obtained for the various TAPS concentrations tested (curves b – h, Figure 4-1), a slight departure from the curve recorded without ligand was observed for all TAPS concentrations tested in the potential range between around 0.1 and -0.6 V. Specifically, in the potential region where copper is reduced (region between 0.1 to -0.2 V), a maximum variation in the capacitive current of 9.5% was recorded for 3.9×10^{-3} M of TAPS. In a previous chapter, similar adsorption interference was obtained with TAPSO (see Part II, Chapter 2, Section 2.3.2), which is a structural related pH buffer. However, no significant influence in the recorded polarograms or in the refined stability constants was verified (see Part II, Chapter 2, Section 2.3.2). Thus, it was decided to use 3.9×10^{-3} M TAPS as the highest ligand concentration for the polarographic study of $\text{Cu}-(\text{TAPS})_x-(\text{OH})_y$ system.

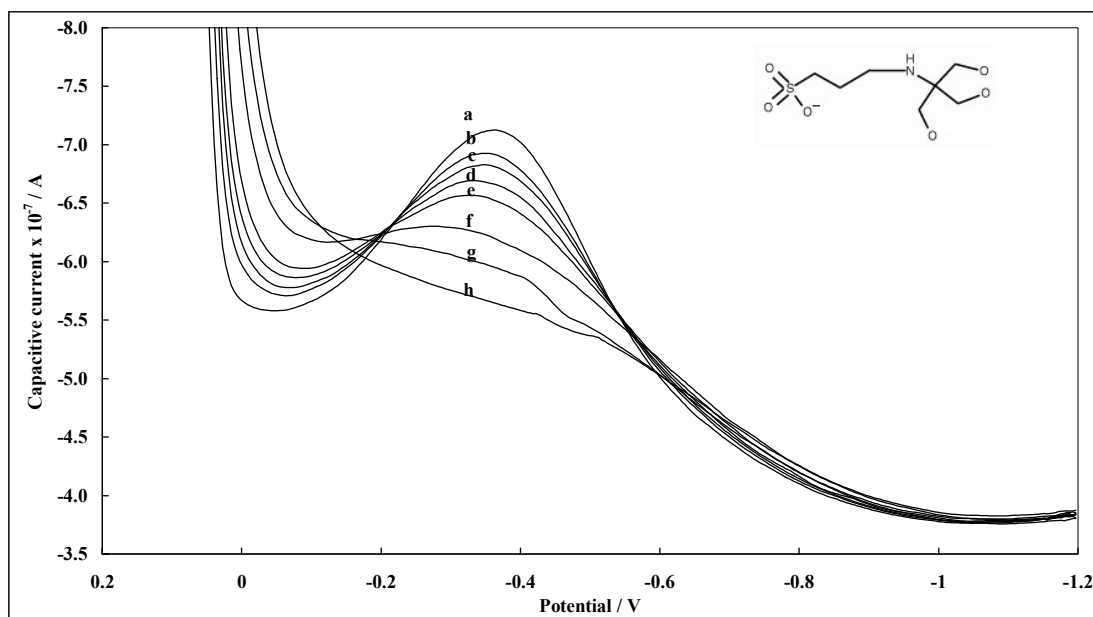


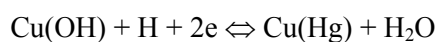
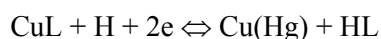
Figure 4-1 AC polarograms for TAPS solutions in 0.1 M KNO₃ at pH = 10.34. [TAPS] = 0.0 (a), 0.8 (b), 1.2 (c), 2.0 (d), 2.9 (e), 5.6 (f), 8.5 (g) and 12.1 × 10⁻³ M (h). The insert in figure represents the structure form of the ligand at pH 10.34, L⁻.

To characterize the Cu-(TAPS)_x-(OH)_y system, two polarographic titrations were performed, [L_T]:[Cu_T] = 151.6 and 97.5 with total copper concentrations of 7.0 × 10⁻⁶ and 4.0 × 10⁻⁵ M, respectively. After the addition of the ligand, the recorded diffusion current of DC wave decreased by about 20 % and the position of the polarographic signal has changed by about 6 mV towards more negative values for the lowest [L_T]:[Cu_T] ratio. For the highest [L_T]:[Cu_T] ratio, a decrease of 13% in the diffusion current and a shift of less than one mV towards more cathodic values was observed. This was probably due to some adsorption of the ligand into the mercury electrode surface. To correct for this phenomenon, the values for the diffusion current and half-wave potential for metal in the presence of the ligand were used as reference point for both titrations, since these values were recorded at a pH where no complexation was expected (pH value below 5.0, Figure 4-2A).

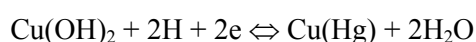
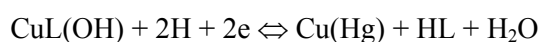
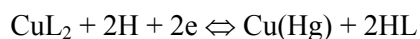
The analysis of the DC polarograms collected for both [L_T]:[Cu_T] ratios, in the pH range between 3.9 – 10.5, showed that Cu-(TAPS)_x-(OH)_y system behaved as labile on the polarographic time scale; only one peak corresponding to all labile species present in solution, which shifted to more cathodic potential values, was observed (data not shown). It was also verified that the system was fully reversible; for both titrations, the steepness of the waves varied between 0.92 and 1.09.

4.3.3 PREDICTION OF SPECIES FORMED FROM DCP TITRATIONS

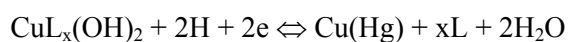
The prediction of the species present in solution was accomplished by graphical analysis of the slopes drawn from the observed half-wave potential shift as a function of pH (Figure 4-2A) and as a function of the logarithm of the free ligand concentration (Figure 4-2B). The results of the two polarographic titrations, $[L_T]:[Cu_T] = 151.6$ (asterisks) and 97.5 (points) with total copper concentrations of 7.0×10^{-6} and 4.0×10^{-5} M, respectively, were included in Figure 4-2, as well as the protonation constant value and the respective forms of the ligand. For the lowest $[L_T]:[Cu_T]$ ratio, the analysis of the data shows that no complexation occurs until pH 5.5 and for $[L_T]:[Cu_T] = 151.6$, the complexation only starts at pH 6.5 (lower metal concentration). For $[L_T]:[Cu_T] = 97.5$, three slopes were identified. Between pH 6.8 and 7.3, a slope of about 30 mV/pH was observed indicating the presence of CuL and/or Cu(OH); one proton is involved to generate HL and/or H₂O in the electrochemical reaction at the dropping mercury electrode (for simplicity, charges were omitted):



Between pH 7.4 and 8.4, a slope of 59 mV/pH was drawn, which indicates that up to three species can be present in solution; CuL₂ and/or CuL(OH) and/or Cu(OH)₂ develop a theoretical slope of 59 mV/pH due to the involvement of two protons in the reaction at the DME:



The formation of Cu(OH)₂, as major species, is very unlikely since no precipitation was observed up to pH 10.5, which shows that copper – TAPS complexes must be formed. From pH 8.5 to 10.5, about the same slope, as that obtained in the pH range between pH 7.4 and 8.4, was obtained, 60 mV/pH. Above the pK_a, the main form of the ligand is L⁻, which indicates the formation of CuL_x(OH)₂ species. The electrochemical reaction at the DME involves two protons:



Apparently, the CuL₂(OH) species does not exist in appreciable amount, under the experimental conditions tested, since no slope indicative of its predominance was obtained.

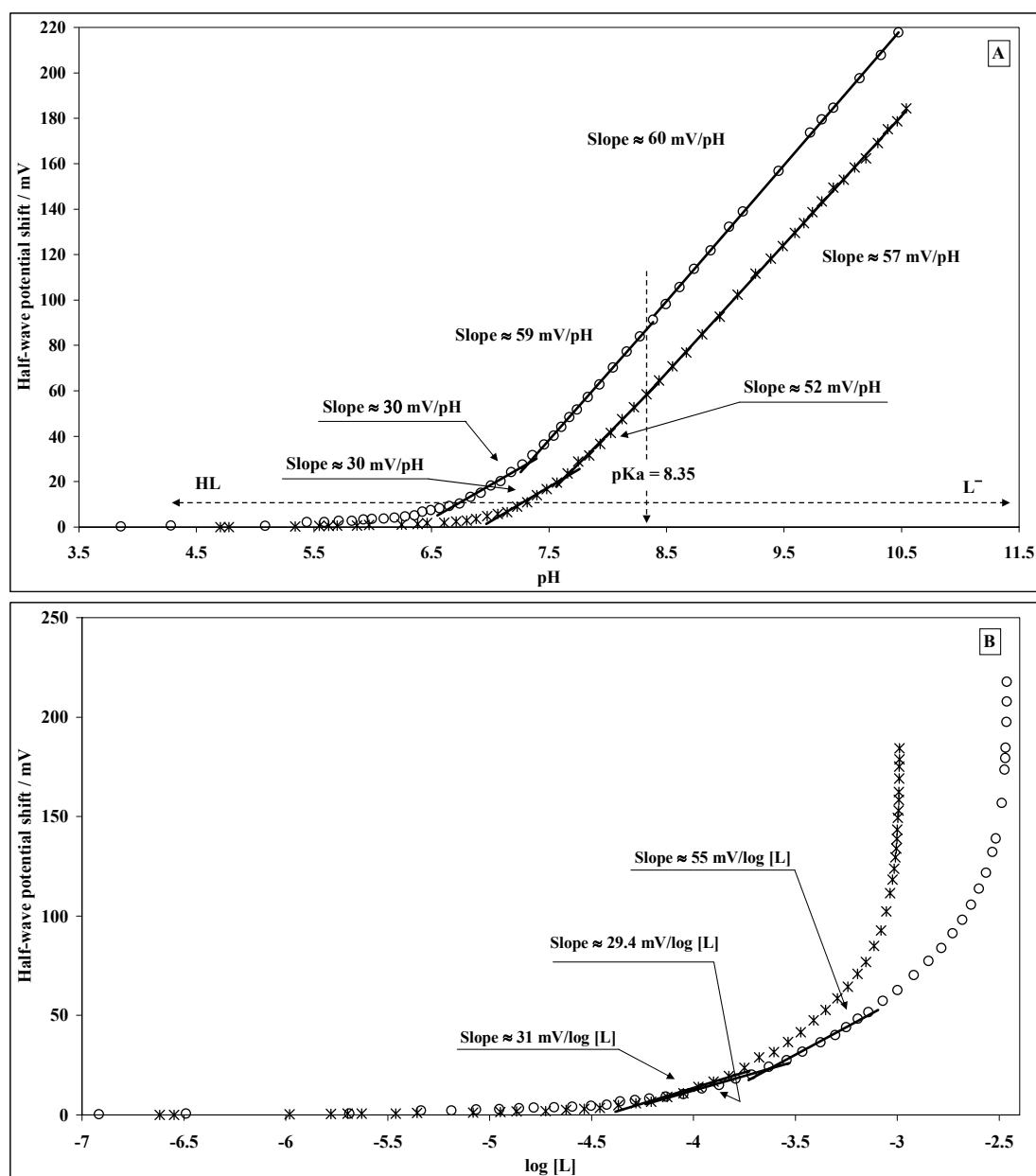


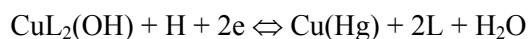
Figure 4-2 Prediction of species for Cu-(TAPS)_x-(OH)_y system, from DCP data: [L_T]:[Cu_T] = 97.5 (points), [L_T]:[Cu_T] = 151.6 (asterisks) with [Cu²⁺] = 4.0 × 10⁻⁵ and 7.0 × 10⁻⁶M, respectively. Observed half-wave potential shift as a function of pH (A) and as a function of the logarithm of the free ligand concentration (B).

If this species was formed below the pK_a, the theoretical slope should be 88.5mV/pH, three protons involved in the reduction reaction at the DME:



and the observed slope was just about 59 mV/pH. But if one considers that this species should exist in higher amount above the pK_a, where the ligand is mainly in the deprotonated form, the slope should

decrease to a value of 29.5mV/pH, only one proton involved in the DME reaction:



So, two possibilities can be considered: i) this species does not exist in appreciable amount in the pH region analysed or ii) is exactly formed in the deprotonation region, which would result in a slope value between 88.5 and 29.5, *i.e.* a slope characteristic of a reduction reaction involving two protons, 59 mV/pH. For [L_T]:[Cu_T] = 151.6, a similar slope profile to that recorded for [L_T]:[Cu_T] = 97.5 was drawn (Figure 4-2A), being about 0.5 units shifted to higher pH values, which evidences a slight decrease of complexation for this ratio.

The graphical analysis of half-wave potential shift *vs.* log[L] (Figure 4-2B) shows characteristic slopes of CuL and CuL₂, for [L_T]:[Cu_T] = 97.5, in the pH range 6.5 – 7.3 and 7.4 – 8.0, respectively. For [L_T]:[Cu_T] = 151.6, a slope characteristic of CuL was possible to draw in the pH range between 7.1 and 7.6. These slopes indicate that these species should be major species in the pH range where the slopes were drawn.

From the considerations described above, the proposed model for Cu-(TAPS)_x-(OH)_y system should include the following species: CuL, CuL₂, CuL(OH) and CuL_x(OH)₂. In order to form a logical sequence of species, the CuL₂(OH) species should also be considered, as a minor species, resulting in the model: CuL, CuL(OH), CuL(OH)₂, CuL₂, CuL₂(OH) and CuL₂(OH)₂.

4.3.4 MODELLING PROCEDURES AND REFINEMENT OF STABILITY CONSTANTS

4.3.4.1 DCP RESULTS

The adopted strategy for the refinement of the stability constants from DCP data from both titrations performed is summarized in Table 4-2. For both [L_T]:[Cu_T] ratios, the refinement operations started with the fitting of two simpler models: Model 1: CuL, CuL₂, CuL(OH) and CuL(OH)₂ and Model 2: CuL, CuL₂, CuL₂(OH) and CuL₂(OH)₂. Because graphical analysis (Figure 4-2B) has confirmed the presence of CuL₂, for [L_T]:[Cu_T] = 97.5, it was decided to include this species in both models. For both [L_T]:[Cu_T] ratios, the fit of Model 1 resulted in the rejection of CuL₂ and the fit of Model 2 improved significantly the standard deviation (Table 4-2), indicating that CuL, CuL₂, CuL₂(OH) and CuL₂(OH)₂ species should exist in considerable amount in the pH range tested. From a statistical point of view, no other species are necessary to explain the experimental data obtained. Given that CuL₂ exists in significant amount, as it is indicated for the lower [L_T]:[Cu_T] ratio (see Figure 4-2B), it is reasonable to consider that the hydroxides species formed in solution result from the hydrolyses of CuL₂ species, explaining the preference for Model 2.

Table 4-2. Overall stability constants for Cu-(TAPS)_x-(OH)_y system determined by DCP: [L_T]:[Cu_T] = 97.5 (49 points between pH 3.9 and 10.5) and 151.6 (52 points between pH 4.7 and 10.5), with [Cu²⁺] = 4.0 × 10⁻⁵ and 7.0 × 10⁻⁶ M, respectively; and GEP: [L_T]:[Cu_T] = 10.0, [Cu²⁺] = 1.0 × 10⁻³ M (four titrations, 118 points between pH 3.4 and 6.6).

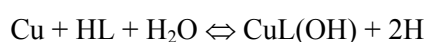
Complexes	DCP				GEP	
	[L _T]:[Cu _T] = 97.5		[L _T]:[Cu _T] = 151.6		[L _T]:[Cu _T] = 10.0	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
CuL Cu + L ⇌ CuL	4.07 ± 0.03	4.10 ± 0.03	4.09 ± 0.05	4.22 ± 0.05	4.10 ± 0.02	4.09 ± 0.02
CuL(OH) Cu + L + OH ⇌ CuL(OH)	10.66 ± 0.03	NI	10.60 ± 0.03	NI	11.05 ± 0.02	NI
CuL(OH)₂ Cu + L + 2OH ⇌ CuL(OH) ₂	16.47 ± 0.01	NI	16.00 ± 0.01	NI	NI	NI
CuL₂ Cu + 2L ⇌ CuL ₂	R	7.58 ± 0.06	R	7.9 ± 0.1	R	7.67 ± 0.02
CuL₂(OH) Cu + 2L + OH ⇌ CuL ₂ (OH)	NI	13.70 ± 0.03	NI	14.01 ± 0.03	NI	NI
CuL₂(OH)₂ Cu + 2L + 2OH ⇌ CuL ₂ (OH) ₂	NI	18.91 ± 0.01	NI	18.97 ± 0.01	NI	NI
SD / mV	0.5841	0.5211	0.5866	0.3326		
Hamilton R-factor					0.06694	0.06882

For DCP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (see Part II, Chapter 2, Section 2.3.3). For GEP, Hamilton R-factor stands for a statistical Hamilton R-factor generated by the program ESTA (see Part V, Chapter 10, Section 10.2.4.1). R – rejected; NI – not included

4.3.4.2 GEP RESULTS

In previous works (Part II, Chapter 2 and [37]), the formation of CuL(OH)_y for Cu-(TAPSO)_x-(OH)_y and Cu-(DIPSO)_x-(OH)_y systems was evidenced particularly when low [L_T]:[Cu_T] ratios were used. In order to design the best experimental conditions to obtain further information about the final model of Cu-(TAPS)_x-(OH)_y system, several SDD were simulated for a wide range of [L_T]:[Cu_T] ratios (between 1 and 20) (data not shown), using Model 2 refined from DCP data (Table 4-2). We were particularly interested on designing experimental conditions for running potentiometric titrations with lower [L_T]:[Cu_T] ratios using high copper concentrations (about 1.0×10^{-3} M). These conditions would favor the formation of CuL(OH)_y species ([37], Part II, Chapter 2) and, if they were formed, would allow us to confirm their presence. For [L_T]:[Cu_T] ratios lower than 17 ([Cu²⁺] = 1.0×10^{-3} M), Cu(OH)₂ precipitation was predicted at about pH 8. For [L_T]:[Cu_T] ratios higher than 18, no precipitation was predicted but $\frac{[L]}{\left[\sum_{x,y=0}^2 \text{CuL}_x(\text{OH})_y\right]}$ ratios higher than 6 were predicted; thus, under these experimental conditions, erroneous stability constants should be obtained because all the titration goes towards the deprotonation of the ligand and not to neutralise protons liberated from the copper complexation. The main reason why for TAPSO and DIPSO was possible to run potentiometric titrations using low [L_T]:[Cu_T] ratios is due to the fact that the amino group of these buffers ([37], Part II, Chapter 2) is more acidic than TAPS' amino group (for TAPSO and DIPSO, the pK_{a2} values are around 7.5, which is about one log unit lower than TAPS - Table 4-1), which gave, for the same experimental conditions, a large amount of free ligand concentration available in the pH range of copper hydrolysis and Cu(OH)₂ precipitation to complex copper and thus avoids its precipitation.

In order to confirm some of the stability constants values (CuL and CuL₂) refined by DCP, it was decided to run potentiometric titrations for [L_T]:[Cu_T] ratio set to 10.0 ([Cu_T] = 1.0×10^{-3} M). Due to the early precipitation, experimental data were only possible to be collected up to pH 6.6. This fact disabled the refinement of CuL_x(OH)₂ species, which should start to be formed at about pH 7.0-7.5. The obtained results are summarized in Table 4-2. Thus, we started with two models: Model 1 constituted by CuL, CuL(OH) and CuL₂ and Model 2 constituted by CuL and CuL₂. Other species (CuL₂(OH) and CuL_x(OH)₂) were not included due to the narrow titration pH range used in the refinement. For Model 1, ESTA rejected CuL₂. However, this cannot really mean that this species was not formed. One must not forget that the formation of CuL₂ and CuL(OH) uses the same amount of protons (for sake of simplicity, charges were omitted):



which makes it difficult for ESTA to distinguish between these two species. Table 4-2 evidences that both final models (Model 1: CuL and CuL(OH) and Model 2: CuL and CuL₂) fitted the experimental results with similar Hamilton R-factors. Additionally, the stability constants values for CuL and CuL₂ species refined by GEP (Model 2) were in perfect agreement with the values refined by DCP data (Model 2, Table 4-2).

4.3.5 FINAL MODEL AND OVERALL STABILITY CONSTANTS VALUES FOR Cu-(TAPS)_x-(OH)_y SYSTEM

The analysis of refinement DCP data evidenced that Model 2 (CuL, CuL₂, CuL₂(OH) and CuL₂(OH)₂) is the model that better explains the experimental data. Additionally, the analysis of GEP data raised the possibility of formation of CuL(OH) species. Considering this, SDD were generated for DCP and GEP experiments using the model constituted by: CuL, CuL(OH), CuL₂, CuL₂(OH) and CuL₂(OH)₂, for which stability constants values were calculated as the average stability constant values (CuL = 4.2, CuL₂ = 7.8, CuL₂(OH) = 13.9 and CuL₂(OH)₂ = 18.9) obtained from all the experiments (two DCP and four GEP experiments) plus the CuL(OH) species refined from GEP (CuL(OH) = 11.05). In Figure 4-3A, the SDD for [L_T]:[Cu_T] ratio 97.5 was included. The species distribution shown in this figure does not corroborate the slopes analysis, hence the major species between 7.0 and 8.0 is CuL(OH) and not CuL₂, as it was evidenced from Figure 4-2B. Thus, if CuL(OH) exists must have a lower log β. Decreasing log β_{CuL(OH)} to 10.6 and maintaining all the other stability constant values (CuL = 4.2, CuL₂ = 7.8, CuL₂(OH) = 13.9 and CuL₂(OH)₂ = 18.9), SDD generated for GEP experimental conditions (Figure 4-3B) indicates that up to pH 6.6, besides the formation of CuL, CuL₂ predominates against CuL(OH) species and even if it was formed there was no sufficient amount to be determined by the GEP tested conditions. Therefore, it is clear that CuL(OH) species is not formed and should not be included in the final model for Cu-(TAPS)_x-(OH)_y system.

Thus, the final model should be CuL, CuL₂, CuL₂(OH) and CuL₂(OH)₂, for which the average stability constant values (CuL = 4.2; CuL₂ = 7.8, CuL₂(OH) = 13.9 and CuL₂(OH)₂ = 18.94) were calculated. SDD were generated for both DCP [L_T]:[Cu_T] ratios, as well as for GEP conditions (Figure 4-4). The analysis of SDD for [L_T]:[Cu_T] ratio 10.0 (Figure 4-4A) predicts the formation of CuL and CuL₂ prior to the precipitation predicted to occur at about pH 6.6, and this precipitation was just observed experimentally. Figure 4-4B and Figure 4-4C evidences that CuL, CuL₂, CuL₂(OH) and CuL₂(OH)₂ and CuL, CuL₂(OH) and CuL₂(OH)₂ are the major species for [L_T]:[Cu_T] ratios 97.5 and 151.6, respectively, whose predominance is in perfect agreement with the graphical analysis slopes (Figure 4-2).

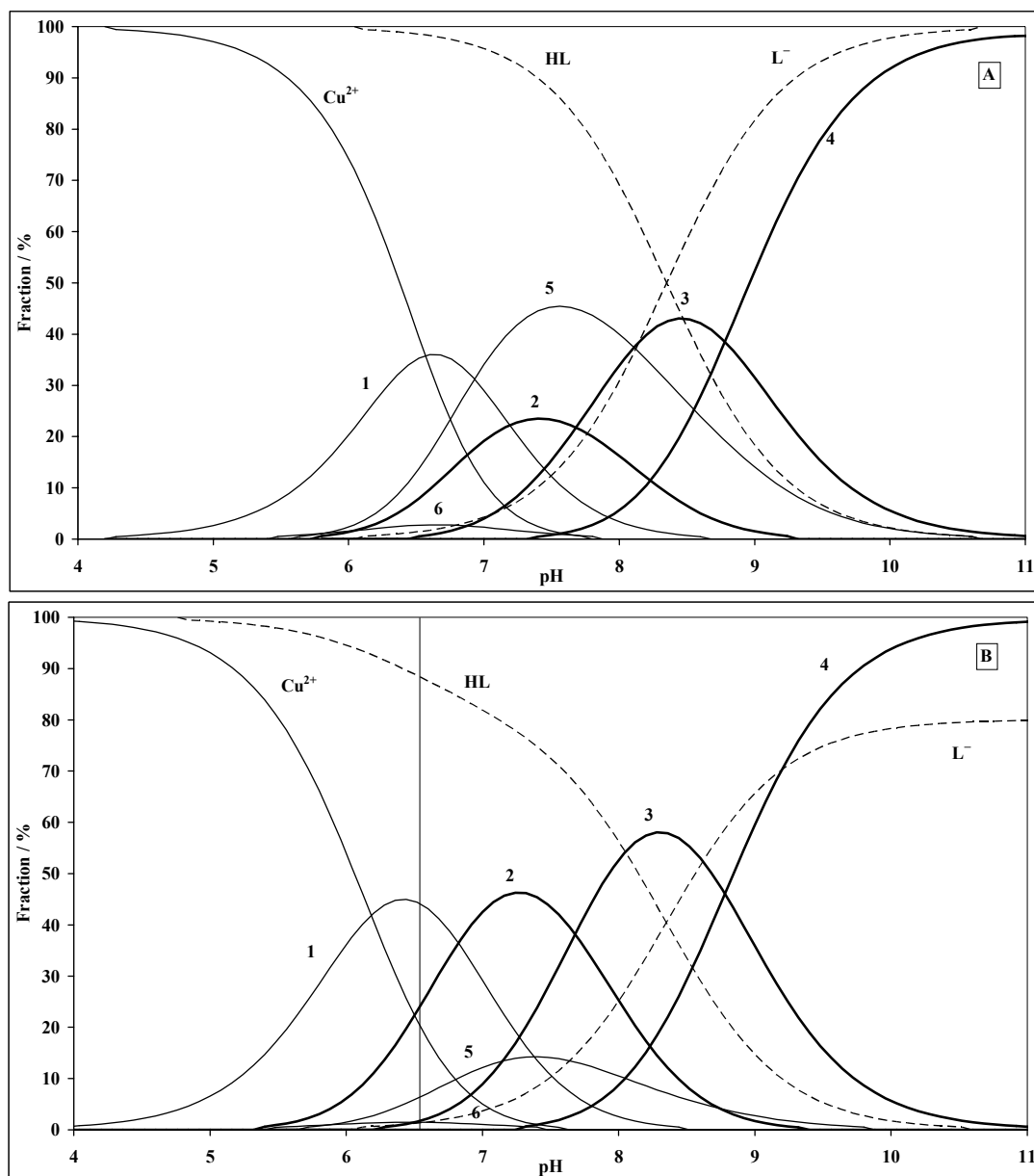


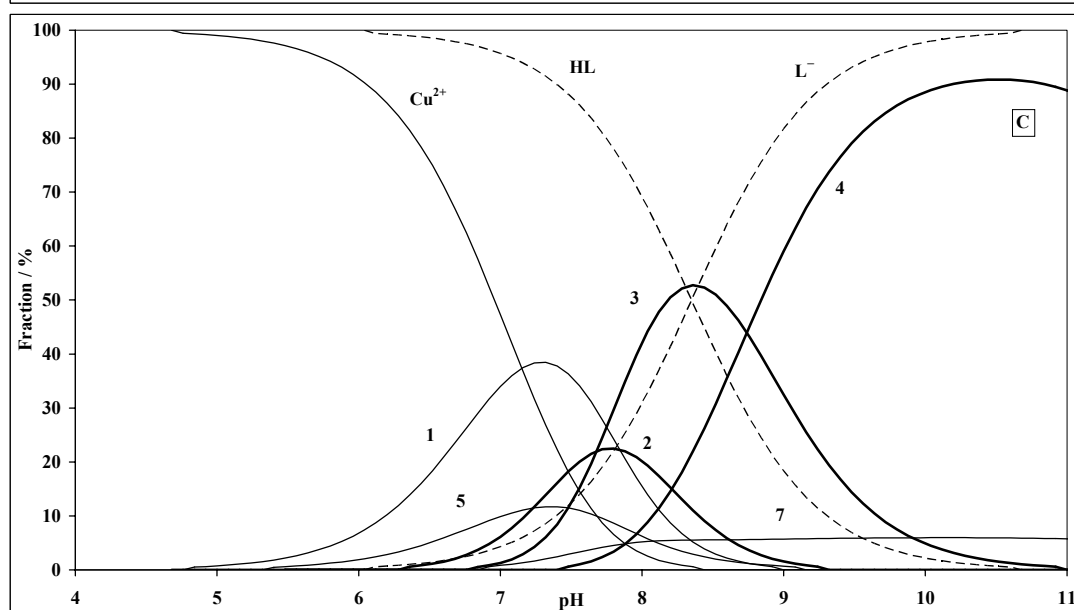
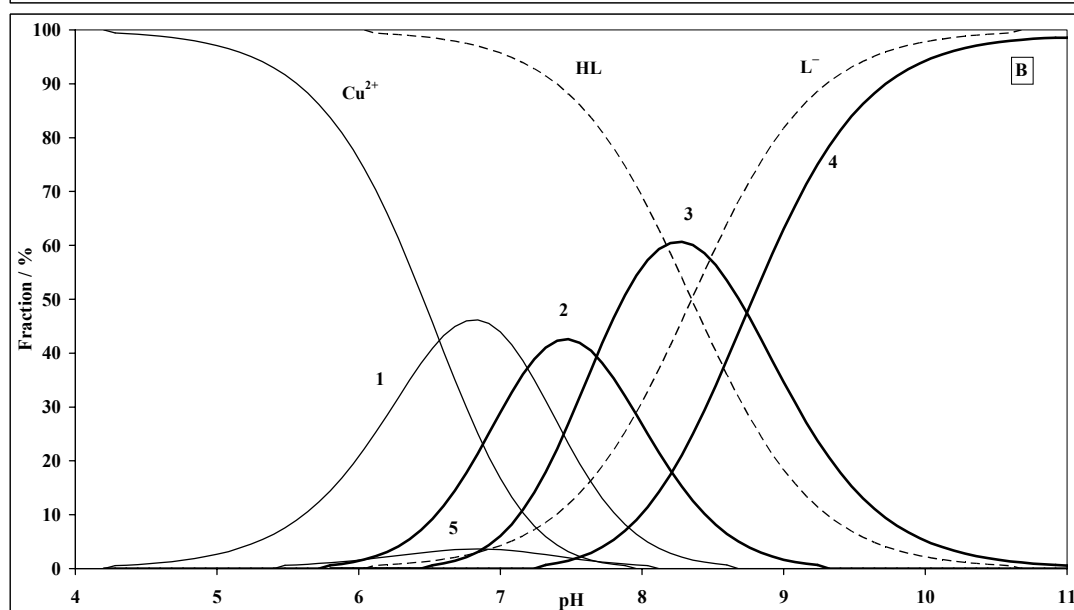
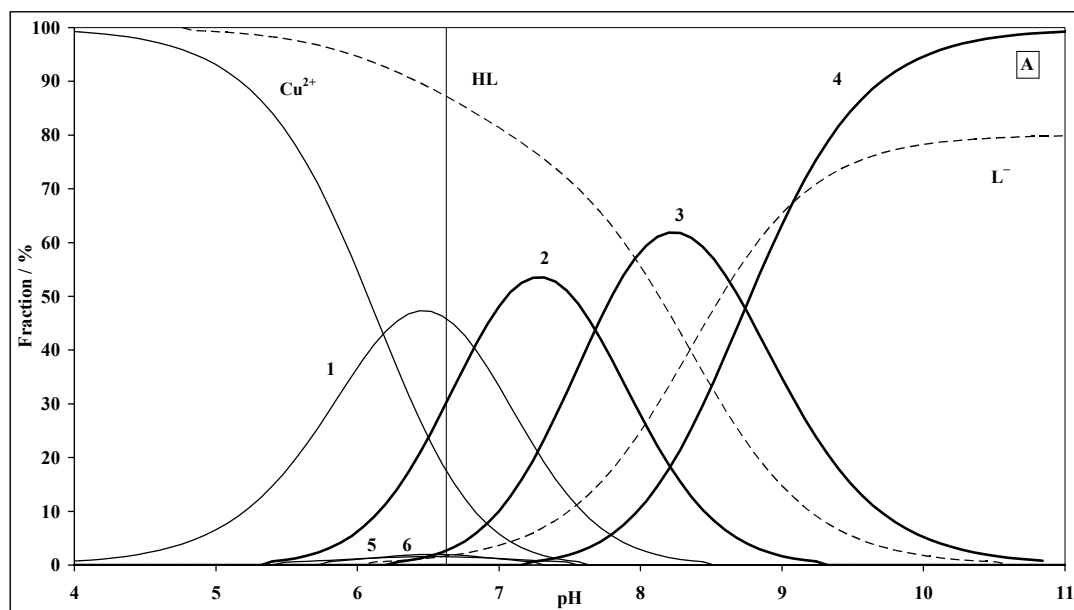
Figure 4-3 Species distribution diagram for $\text{Cu}-(\text{TAPS})_x-(\text{OH})_y$ system. (A) $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 97.5$, $[\text{Cu}^{2+}] = 4.0 \times 10^{-5} \text{ M}$; (B) $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 10$, $[\text{Cu}^{2+}] = 1.0 \times 10^{-3} \text{ M}$. The species present in the figure are: 1 - CuL , 2 - CuL_2 , 3 - $\text{CuL}_2(\text{OH})$, 4 - $\text{CuL}_2(\text{OH})_2$, 5 - $\text{CuL}(\text{OH})$, and 6 - $\text{Cu}(\text{OH})$, with stability constants set to (as $\log \beta$): 4.2, 7.8, 13.9, 18.9, 11.05 (A) or 10.6 (B), and 6.1, respectively. The vertical line indicates the pH at which precipitation was observed.

As suspected by the prediction of species (Figure 4-2), $\text{CuL}_2(\text{OH})$ is formed exactly in the region of the deprotonation of the ligand and this is why no characteristic slope for this species was found in the graphical analysis (Figure 4-2B); the obtained slope resulted from the average of the predicted theoretical slopes prior and after deprotonation (see Section 4.3.3). Above pH 9.0 $\text{CuL}_2(\text{OH})_2$ is the major species present in solution. These SDD (Figure 4-4) also show that, in the buffering pH-range

(between 7.34 and 9.34), $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ are the main species present in solution.

It is very important to emphasize that the characterization of $\text{Cu}-(\text{TAPS})_x-(\text{OH})_y$ system was based in the analysis of a wide-ranging experimental conditions: $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ varied from 10 up to 151.6, with $7.0 \times 10^{-6} < [\text{Cu}^{2+}] < 1.0 \times 10^{-3} \text{ M}$ and $1.1 \times 10^{-3} < [\text{TAPS}] < 1.0 \times 10^{-2} \text{ M}$. Results described above have shown that no other $[\text{L}_\text{T}]:[\text{Cu}_\text{T}]$ ratios were possible to be tested and provide additional information about the final model.

Figure 4-4 Species distribution diagram for the final model of $\text{Cu}-(\text{TAPS})_x-(\text{OH})_y$ system. (A) $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = \rightarrow$ 10, $[\text{Cu}^{2+}] = 1.0 \times 10^{-3} \text{ M}$; (B) $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 97.5$, $[\text{Cu}^{2+}] = 4.0 \times 10^{-5} \text{ M}$; (C) $[\text{L}_\text{T}]:[\text{Cu}_\text{T}] = 151.6$, $[\text{Cu}^{2+}] = 7.0 \times 10^{-6} \text{ M}$. The species present in the figure are: 1 - CuL , 2 - CuL_2 , 3 - $\text{CuL}_2(\text{OH})$, 4 - $\text{CuL}_2(\text{OH})_2$, 5 - $\text{Cu}(\text{OH})$ and 6 - $\text{Cu}_2(\text{OH})_2$, 7 - $\text{Cu}(\text{OH})_2$, with stability constants set to, as $\log \beta$, 4.2, 7.8 13.9, 18.94, 6.1, 16.8 and 11.8, respectively. The vertical line indicates the pH at which precipitation was observed.



4.3.6 COMPARATIVE ANALYSIS OF SEVERAL $\text{Cu}-(\text{L})_x-(\text{OH})_y$ SYSTEMS WITH STRUCTURAL PROPOSAL

As mentioned before, TAPS is a zwitterionic buffer structurally related with others biological buffers, namely, AMPSO, BISTRIS, DIPSO, TAPSO, TES and TRIS, whose structures can be found in Table 4-3. Protonation constants, as well as values for the CuL complex stability constant (as $\log \beta$) for all these $\text{Cu}-(\text{L})_x-(\text{OH})_y$ systems, are also summarized in Table 4-3. Additionally, protonation constants and $\log \beta$ for CuL for Methylamine, Ethanolamine and Ammonia were also included (Table 4-3).

Regarding the basicity of the amino group, it is possible to verify that the substitution of hydroxyl groups in the ligand leads to a systematic decrease of pK_a values; for instance AMPSO, TAPS and TAPSO, which are structural related, have two, three and four hydroxyl groups, respectively, and a decrease of the protonation constant of about 0.7 and 0.8 log units was observed (Table 4-3). In contrast, the extent of the carbon chain increases the pK_a values. While TES has an ethanesulfonic acid chain, TAPS has a propanesulfonic acid and its pK_a value is about 0.8 log units higher than for TES (Table 4-3). The substitution of a methyl group in ammonia results in Methylamine buffer with a pK_a 1.4 log units higher (Table 4-3).

A simple evaluation based on the basicity of the ligands' amino groups, points out to enlargement in the stability constants values with the increase of the pK_a [43], which was observed for TES, TAPS and AMPSO, but for other buffers, this was not the case. To account for the different basicity of these ligands, a graphical representation of the $\log \beta_{\text{ML}}$ vs. pK_a values was drawn (Figure 4-5); with increasing ligand basicity, complex stability should also increase and for structurally related ligands straight lines should result [44].

Table 4-3 Comparison between CuL complex stability constants and basicity for some biological buffers and other amines.

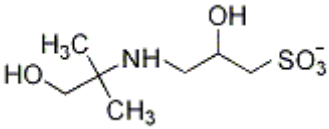
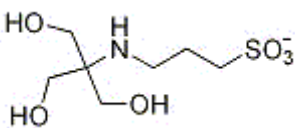
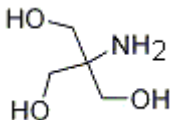
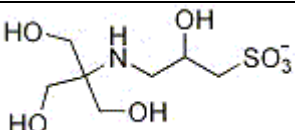
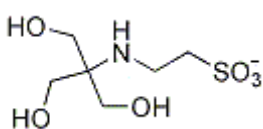
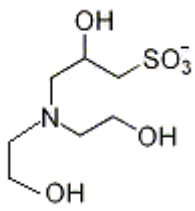
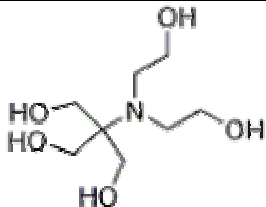
Ligand	Structures	pK_a	$\text{Log}\beta_{\text{CuL}}$	References
Methylamine	H_2NCH_3	10.65	4.11	[16]
Ethanolamine	$\text{H}_2\text{NCH}_2\text{CH}_2\text{OH}$	9.52	4.50	[16]
Ammonia	NH_3	9.28	4.12	[16]
AMPSO		9.05	4.62	Chapter 3
TAPS		8.34	4.2	This work
TRIS		8.10	4.05	[16]
TAPSO		7.55	4.41	Chapter 2
TES		7.5	3.9	[16]
DIPSO		7.47	4.2	[37]
BIS-TRIS		6.54	5.27	[16]

Figure 4-5 shows that TES, TRIS, TAPS, AMPSO fitted rather well to a straight line, indicating that with the increase of their pK_a values a linear increase of the stability constant was observed. Below that straight line, it is possible to find the stability constants for Ammonia, Ethanolamine and Methylamine.

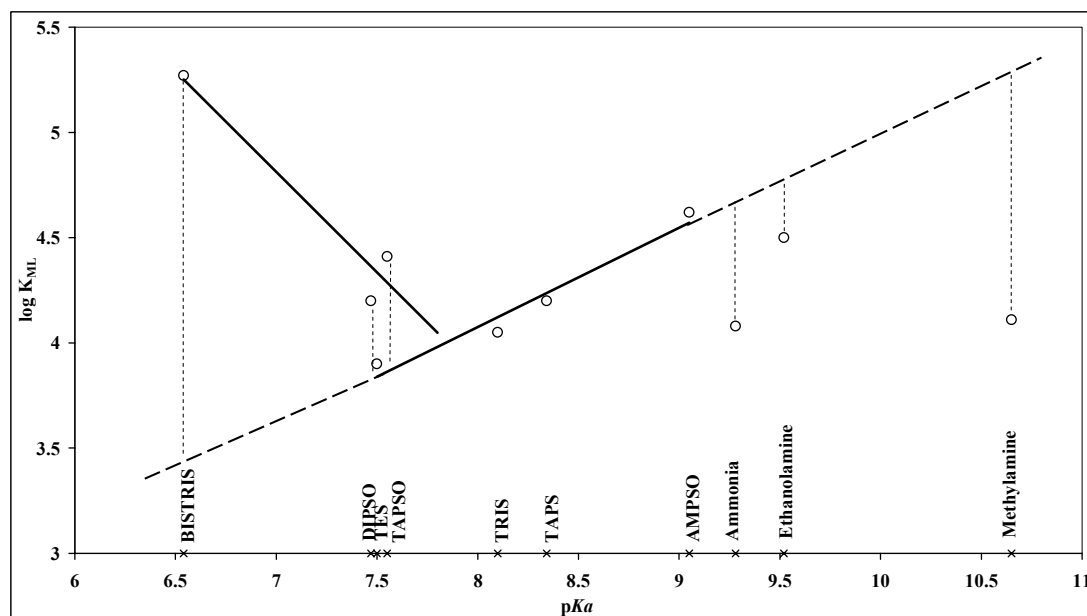


Figure 4-5 Relationship between $\log \beta_{CuL}$ and pK_a for Cu^{2+} and several biological buffers and other amines.

From these three ligands, Ethanolamine is closer to the straight line (Figure 4-5), which is a hint that one hydroxyl group of TES, TRIS, TAPS, AMPSO and Ethanolamine, participate in the complex formation, forming a five-membered chelate ring. This is in agreement with the fact that crystal structures of Cu^{2+} complexes with TRIS, determined by single-crystal X-ray data, evidenced that TRIS ligand coordinated via amino and hydroxymethyl groups forming a five-membered chelate ring [45, 46]. Figure 4-5 also evidenced that BISTRIS, DIPSO and TAPSO are above the straight line, indicating that the steric hindrance is diminished, by a stronger chelating effect. It is also possible to draw a second straight line with a negative slope with BISTRIS, DIPSO and TAPSO, as it is shown in Figure 4-5. This line represents an increase of the chelating effect hence with the increase of pK_a the $\log \beta_{CuL}$ diminished (Figure 4-5). This fact is probably due to additional formation of one or more five-membered chelate rings involving additional hydroxyl groups. Recently, the analysis of crystal Cu^{2+} -BIS-TRIS, by single crystal X-ray diffraction analysis, evidenced that BIS-TRIS acted as a neutral pentadentate ligand: four hydroxyl oxygen atoms and one nitrogen atom [47].

The comparative analysis of the strength of $Cu(TAPSO)^+$ and $Cu(TAPS)^+$ (Figure 4-5) with the

structures of the ligands (Table 4-3) suggests that the TAPSO's hydroxyl group near the sulfonic group is also involved in the coordination, forming the second additional five-membered chelate ring and increasing the stability of the CuL complex. Thus, TAPSO is most likely a tridentate ligand, coordinating by the amino group and by two hydroxyl groups, forming two five-membered chelate rings. On the other hand, TAPS is most likely a bidentate ligand coordinating via amino and one of the β -hydroxy groups forming one five-membered chelate ring. The proposed structures for $\text{Cu}(\text{TAPSO})^+$ and $\text{Cu}(\text{TAPS})^+$ complexes are illustrated in Figure 4-6.

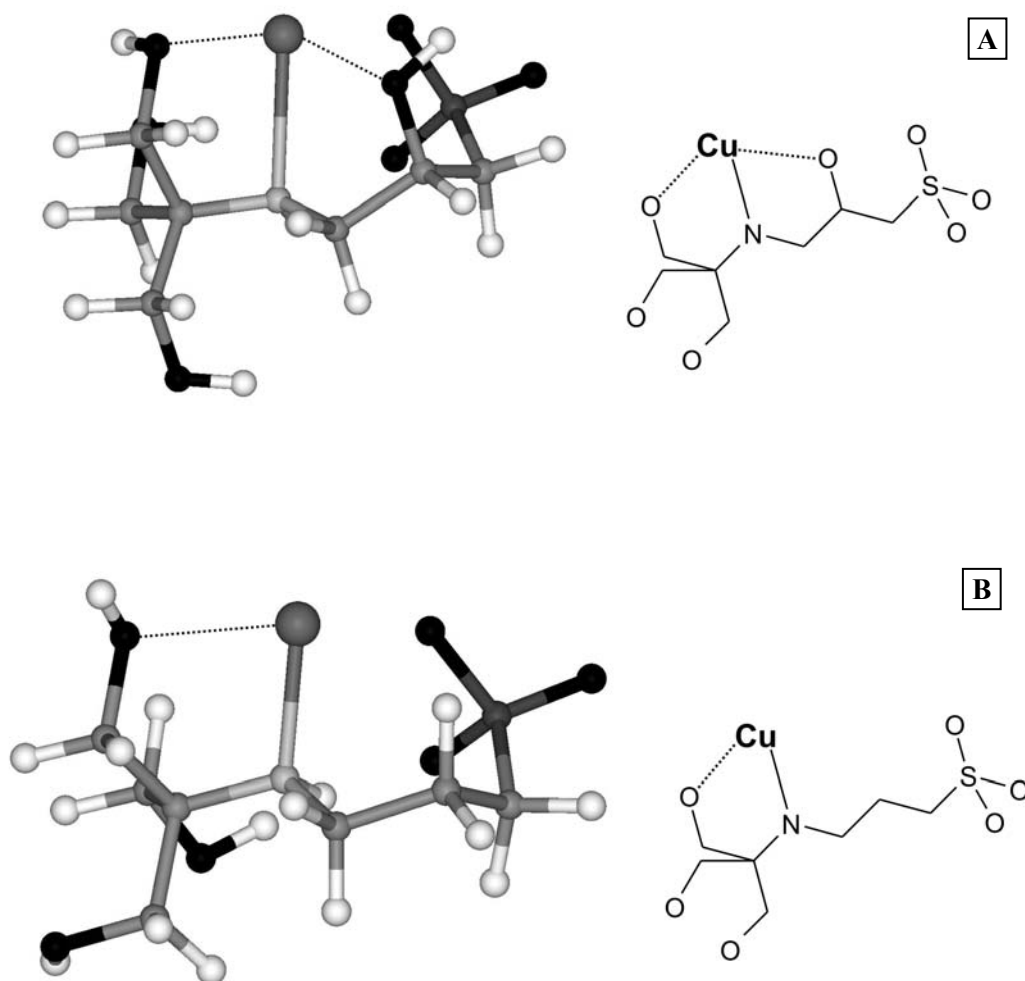


Figure 4-6 Proposed structures for $\text{Cu}(\text{TAPSO})^+$ (A) and $\text{Cu}(\text{TAPS})^+$ (B) complexes. For $\text{Cu}(\text{TAPSO})^+$ complex, copper coordination to TAPSO' amino group and two hydroxyl groups forming two five-membered chelate rings is shown (A). For $\text{Cu}(\text{TAPS})^+$ complex, the coordination via amino and one of the β -hydroxy groups of TAPS forming only one five-membered chelate ring (B) is proposed.

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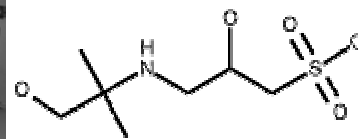
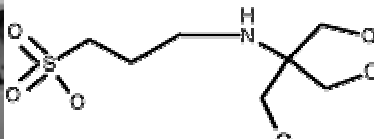
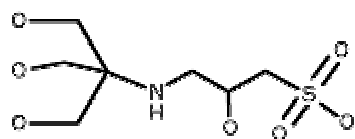
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PART III MODELLING OF $Pb-(BUFFER)_X-(OH)_Y$ SYSTEMS

5 GRAPHIC DATA ANALYSIS AND COMPLEX FORMATION CURVES AS MODELLING AND OPTIMISATION TOOLS IN POTENTIOMETRIC AND VOLTAMMETRIC SPECIATION STUDIES OF A $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ SYSTEM⁴

ABSTRACT

The formation of complex species between lead and TAPSO, a commercialised biological buffer, was evidenced and discussed. Formation constants of $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system have been studied by direct current polarography and by glass electrode potentiometry at fixed total–ligand and total–metal concentration ratio and varied pH, at 25.0 °C and ionic strength set to 0.1 M KNO_3 . The graphic analysis revealed to be a very powerful tool in the prediction and refinement operations of $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system. The proposed final model for this system is: PbL , PbL_2 , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$, with stability constants values, as $\log \beta$, of 3.7 ± 0.1 , 6.6 ± 0.1 , 12.9 ± 0.1 and 17.8 ± 0.1 , respectively.

5.1 INTRODUCTION

Some biological and biochemical studies as well as heavy metals speciation studies performed on environmental samples usually require the pH control.

In the last three decades, Good and co-workers have synthesized several buffers covering the pK_a range of 6.15 to 8.35 [1, 2]. These buffers are zwitterionic aliphatic amines that are either substituted glycines or substituted taurines. In the 80's years, Ferguson and co-workers synthesised more substituted amino sulfonic acids buffers [3].

One of the intended characteristics of Good's buffers was to assure that these buffers do not complex with metals. However, when these buffers are used in studies involving metals ions in solution, it is very important to consider the possible interference of them, due to the complexation with metals. If complexes are formed, then stability constants must be known. Good buffers are widely used in biological, biochemical and environmental studies, but unfortunately in some cases there is deficient information related to theirs thermodynamics, namely complex stability constants.

In a previous work, the influence of TAPSO in systems with copper metal ion was analysed (Part

⁴ Electroanalysis 17 (2005) 1291-1301

II, Chapter 2). An important lesson learned from this study was that copper forms strong complexes with TAPSO in its buffer pH range. When these type of compounds, such as is the case of TAPSO, are employed as a buffer then its concentration is very large (about 10^{-3} and 0.1 M). Consequently, the addition of buffer may strongly disturb the metal mass–balance of the system under study and thus modify the answer of biochemical/environmental reactions containing these metal ions. So, the knowledge of the metal stability constants allows us to define correctly the metal speciation for a specific system.

TAPSO is a zwitterionic buffer, commercially available from Sigma – Aldrich, whose protonation constant is near neutrality ($pK_a = 7.55$ (Part II, Chapter 2)). However, besides its pK_a value and copper stability constants (Part II, Chapter 2), no information is provided on its complexing abilities with lead ion. Additional information of the metal stability constants with TAPSO will allow a properly characterization of this buffer. These values can then be entered as “known” in the models.

The aim of the present work was the study of complex formation equilibria of TAPSO with lead(II) ion. The work was performed by using different electrochemical techniques: DCP, DPP and GEP, at fixed total ligand to total lead ion concentration ($[L_T]:[Pb_T]$) ratios and various pH values, at 25.0 °C and 0.1M KNO_3 ionic strength.

The use of $[L_T]:[Pb_T]$ ratios of various orders of magnitude, and thus total lead ion concentration, allowed us to characterise properly the final model, which is reported here for the first time.

Experiments by ACP were also carried out to check buffer adsorption at the mercury working electrode surface, under the experimental conditions used.

5.2 EXPERIMENTAL

5.2.1 MATERIALS AND REAGENTS

The stock solution of lead, 5.0×10^{-2} M was prepared from the analytical grade salt ($Pb(NO_3)_2$, Merck). It was acidified with standardized nitric acid to pH 3.0 and the ionic strength was adjusted to 0.1 M. In potentiometry, a Merck standard solution of lead (9.7×10^{-3} M), was used.

Additional information about the remaining reagents and materials were described previously (Part II, Chapter 2, Section 2.2.1).

5.2.2 APPARATUS

All experiments were carried out in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer, and thermostatted at $25.0 \pm 0.1^\circ\text{C}$ using a water bath.

5.2.2.1 POLAROGRAPHY

Polarographic measurements of $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system, was performed as previously described (Part II, Chapter 2, Section 2.2.2.1).

5.2.2.2 POTENTIOMETRY

The potentiometric titration of $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system was performed as previously described (Part II, Chapter 2, Section 2.2.2.2).

5.2.3 PROCEDURE

5.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

For all techniques, the calibration of the glass electrode (measurements of pH as $-\log [\text{H}]$) was accomplished as previously described (Part II, Chapter 3, Section 3.2.3.1).

5.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

In order to evaluate the adsorption of the ligand on the surface of the mercury working electrode, ACP polarograms were run in the absence of the metal ion. The experimental conditions used were described elsewhere (Part II, Chapter 2, Section 2.2.3.2).

5.2.3.3 DIRECT CURRENT AND DIFFERENTIAL PULSE POLAROGRAPHY

The polarographic techniques (DC and DP) were performed on the same solution sample to evaluate the electrochemical behaviour of the $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system. In the DPP measurements, a pulse height of 50 mV was used, with pulse width and integration time of 200 ms and 60 ms,

respectively. A step potential of 4 mV and a drop time of 1 s were used for both techniques (DCP and DPP). Two different $[L_T]:[Pb_T]$ ratios were evaluated, 102 and 197, with $[Pb_T] = 4.5 \times 10^{-5}$ M. The pH of the solution sample was varied in small steps, usually in 0.1 pH units, between pH 5.0 and 10.0 with the addition of standardised base solution and after each addition a new set of polarograms was recorded. 30 to 50 polarograms were recorded in the whole pH range and obtained for each total ligand to total metal ion concentration ratio. The procedure used to collect the data points was identical to the one described previously (Part II, Chapter 2, Section 2.2.3.3). The equilibrium of the metal-ligand solutions was tested and it was reached in a few minutes.

5.2.3.4 GLASS ELECTRODE POTENTIOMETRY

Five potentiometric titrations in the pH-range between 5.0 and 10.0 were performed (about 45 points were collected) to establish the model and determine the stability constants. A $[L_T]:[Pb_T]$ ratio of 10.0 was used with a total lead concentration of 4.8×10^{-4} M. Another set of titrations was performed with a total lead concentration of 5.2×10^{-4} M and a $[L_T]:[Pb_T]$ ratio of 3.3. About 100 points were collected between pH 4.0 and 10.0. Three titrations were performed. For both ratios, monotonic additions of standardised KOH were used.

5.2.4 DATA TREATMENT

The refinement of polarographic data was done using the Cukrowski method [4, 5]; as it was described previously (Part II, Chapter 2, Section 2.2.4).

The analysis of potentiometric data was done with ESTA program [6, 7]. For more details about the program see Part II, Chapter 2, Section 2.3.3 and Part V, Chapter 10, Section 10.2.4.1.

During the refinement of the overall stability constants of $Pb-(TAPSO)_x-(OH)_y$ complexes, the hydrolysis constants of lead, compiled from the literature [8, 9] (Table 5-1), were introduced as fixed parameters, as well as the protonation constants of TAPSO and of water [8] (Part II, Chapter 2, Table 2-1).

Table 5-1 Overall stability constants for Pb²⁺ complexes with OH⁻, at 25 °C.

Equilibrium	Log β	I (M)	References
$\text{Pb}^{2+} + \text{OH}^- \rightleftharpoons \text{Pb}(\text{OH})^+$	6.0	0.1	[8]
$2\text{Pb}^{2+} + \text{OH}^- \rightleftharpoons \text{Pb}_2(\text{OH})^{3+}$	7.8	3.0	[8]
$\text{Pb}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Pb}(\text{OH})_2$	10.3	0.5	[8]
$\text{Pb}^{2+} + 3\text{OH}^- \rightleftharpoons \text{Pb}(\text{OH})_3^-$	13.3	0.5	[8]
$3\text{Pb}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Pb}_3(\text{OH})_4^{2+}$	31.7	0.5	[8]
$4\text{Pb}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Pb}_4(\text{OH})_4^{4+}$	35.5	0.1	[8]
$6\text{Pb}^{2+} + 8\text{OH}^- \rightleftharpoons \text{Pb}_6(\text{OH})_8^{4+}$	67.4	0.5	[8]
$\text{Pb}(\text{OH})_2(\text{s}) \rightleftharpoons \text{Pb}^{2+} + 2\text{OH}^-$	-16.1	0.0	[9]

5.3 RESULTS AND DISCUSSION

5.3.1 LIGAND ADSORPTION AND LEAD REVERSIBILITY STUDIES

The increase of ligand concentration at the mercury surface, compared with the bulk concentration, indicates that the ligand adsorbs at the surface of mercury electrode. Uncharged surfactants have a maximum adsorption in the region of zero charge potential (about -0.4V), *i.e.* the region where the surface of the mercury electrode has no charge, and the amount of adsorption decreases at potentials both more positive and negative [10]. ACP at a phase angle of 90° was used to test if there was adsorption of TAPSO at the mercury electrode surface. By comparing the AC polarograms between the background solution and the polarograms obtained with increasing ligand additions, the adsorption effect can be quantified. In Figure 5-1, the variation of capacitive current obtained with the increase of the ligand concentration relative to the background solution (0.1 M KNO₃), expressed in percentage, is represented for three pH values.

The pH values were chosen taking into account the different forms of the monoprotinated ligand; at pH 5.55, the ligand exists only in its protonated form, HL, at pH 9.55, it exists mainly in the deprotonated form, L⁻, and at pH equal to pK_a value, it exists half in each form. The studies showed that there was a small ligand activity, in the range of potential where lead is reduced, $E = -0.35$ V (Figure 5-1) [11]. The largest variation in the capacitive current was observed for pH = pK_a, where the ligand is half in the deprotonated form and half in the zwitterionic form. However, the difference between the variation in the capacitive current recorded at this pH and pH 9.55, where the ligand is fully deprotonated, is insignificant. This fact suggests that, at this potential, the adsorption of the ligand is mainly due to the uncharged part of the ligand, 2-Hydroxy-3-

[tris(hydroxymethyl)methylamino]-1-propane.

For DCP and DPP experiments, the highest ligand concentration used was 8.8×10^{-3} M. At this concentration, the adsorption studies evidenced a maximum variation in the capacitive current at the applied potential -350 mV of about 10 %, at pH 7.55. However, significant impact neither in the recorded polarograms nor in the refined stability constants was verified, as will be demonstrated throughout the chapter. After the addition of the ligand, the recorded peak or DC wave decreased by about 20 % and the position of the polarographic signal changed by about 2 mV towards more positive values, for both analytical techniques and both ratios. This can possibly be interpreted as an evidence of adsorption of the ligand. To correct for that phenomenon and because the addition of ligand was performed at pH value at which no complexation was expected, the peak potential and peak height (the same applies for the DC wave) after the addition of ligand was taken as a starting (reference) point instead of peak height and position for the metal ion only. Otherwise, one can say that the reduction of a metal ion in the presence of the ligand was used as a reference potential in the computation of the shift in the peak potential.

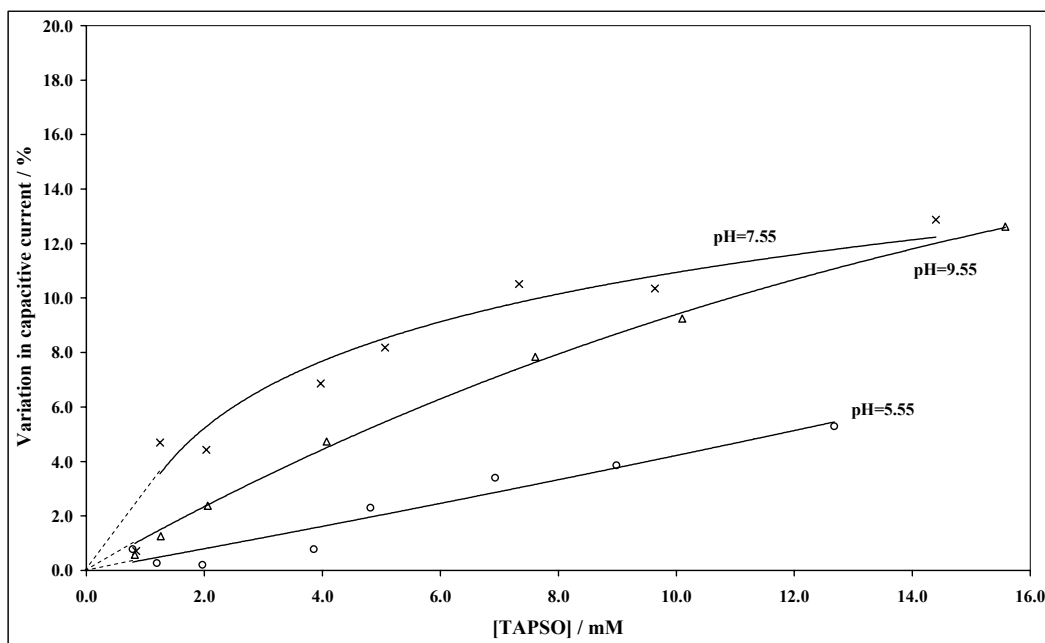


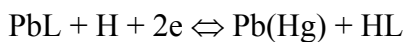
Figure 5-1 Variation of the capacitive current recorded by ACP, without lead ion, with the increase of TAPSO concentration, at three pH values. The values, expressed in percentage, were calculated from the difference between capacitive current values recorded in the absence and in the presence of the buffer, at a potential of -0.35 V, from capacitive current vs. potential ACP curves with a phase angle of 90° in 0.1 M KNO_3 . For other experimental conditions, see Experimental Section.

The analysis of DCP and DPP titrations, performed at different ligand to metal ratios, in a pH range between 5 and 10, showed that $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system behaved as labile on the polarographic time scale; only one peak corresponding to all labile species present in solution, which shifted to more cathodic potential values, was observed (data not shown). The reversibility of the system can be quantified in terms of peak width, $w_{1/2}$ and wave steepness, for DPP and DC techniques, respectively. The DPP peak width (for $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 102$) varied between 61.0 and 69.0 from pH 5.5 until 10.0. A correction for the increase in the irreversibility was done. The peak height was fixed and the peak width kept at a reversible value, 62 mV [12], and the corrected peak potential was obtained. However this correction was not total, still a variation in the peak height is observed according to the variation in the peak width, as is shown in Figure 5-2A.

For pH values between pH 5.5 and 8.0 the $w_{1/2}$ varied between 61.5 and 62.0 and the normalised current follows a normal pattern. Around pH 8.3 an increase in the normalised peak height is observed and this is due to a small decrease in the $w_{1/2}$. Above pH 9.0 the $w_{1/2}$ increases above 62.0 mV resulting in a decrease of the normalised peak current. The analysis of DCP current (Figure 5-2A) shows that there is no drop or increase throughout the experiment, since the limiting current does not depend on the degree of the reversibility of the electrochemical process. This fact stresses the use of DCP technique in cases where the process is not fully reversible. From this point, all the results discussion in this paper will be referred to the DCP technique.

5.3.2 PREDICTION OF SPECIES FORMED FROM DCP TITRATIONS

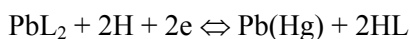
The interpretation of experimental results obtained for the labile metal–ligand system, $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$, is completed with the model of species formed in solution and their stability constants, for which the calculated complex formation curve fits the best the experimental one [5]. This curve fitting procedure begins with good guesses of the species present in solution. The prediction of species was based in graphical analysis of $E_{1/2}$ plotted vs. pH and $E_{1/2}$ plotted vs. $\log [\text{L}]$ and interpretation of the slopes. In Figure 5-2B, a plot of the half-wave potential as a function of pH, for $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ of 102, is shown. Similar results were obtained for the other ratio (data not shown). In this figure, the value of the protonation constant and the respective forms of the ligand are also marked. The analysis of the plot of $E_{1/2}$ vs. pH (Figure 5-2B) shows that until pH 6.4 only a slight complexation occurred ($\Delta E_{1/2} \cong 10$ mV). In region I, pH between 6.4 and 7.4, a slope of $\cong 30$ mV / pH unit was obtained. This fact probably indicates that PbL is the predominant species in this pH range, according to the following electrochemical reaction, in which one proton is involved to generate HL (for sake of simplicity charges were omitted):



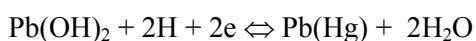
Between pH 7.4 and 8.7, region II, a slope of about 41 mV / pH unit was observed. In this region, the ligand is mainly in the deprotonated form. If the formation of $\text{PbL}_x(\text{OH})$ species only occurs, a theoretical slope of 29.5mV should be obtained (only one proton involved) according to the following electrochemical reaction (in Figure 5-2B the theoretical slope is represented by the dashed line):



However in this pH range there is some amount of HL. PbL_2 can also be formed there and this can explain the increase of slope above 29.5 mV. If PbL_2 is formed then the theoretical Nernstian slope would be 59 mV (two protons involved):



The reduction of the metal hydroxide, Pb(OH)_2 , should also be considered, which involves two protons, *i.e.*:



The formation of the metal hydroxide species as a major one should be excluded since no precipitation was observed up to pH 10, which indicates that the ligand must be involved in the complex formation with lead.

The continuous increase of the slope to a value of about 52 mV indicates the formation of $\text{PbL}_x(\text{OH})_2$ species in region III (pH from 8.7 to 10). The theoretical slope value for the formation of $\text{PbL}_x(\text{OH})_2$ species is 59 mV, due to the involvement of two protons at the DME electrode:

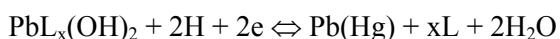
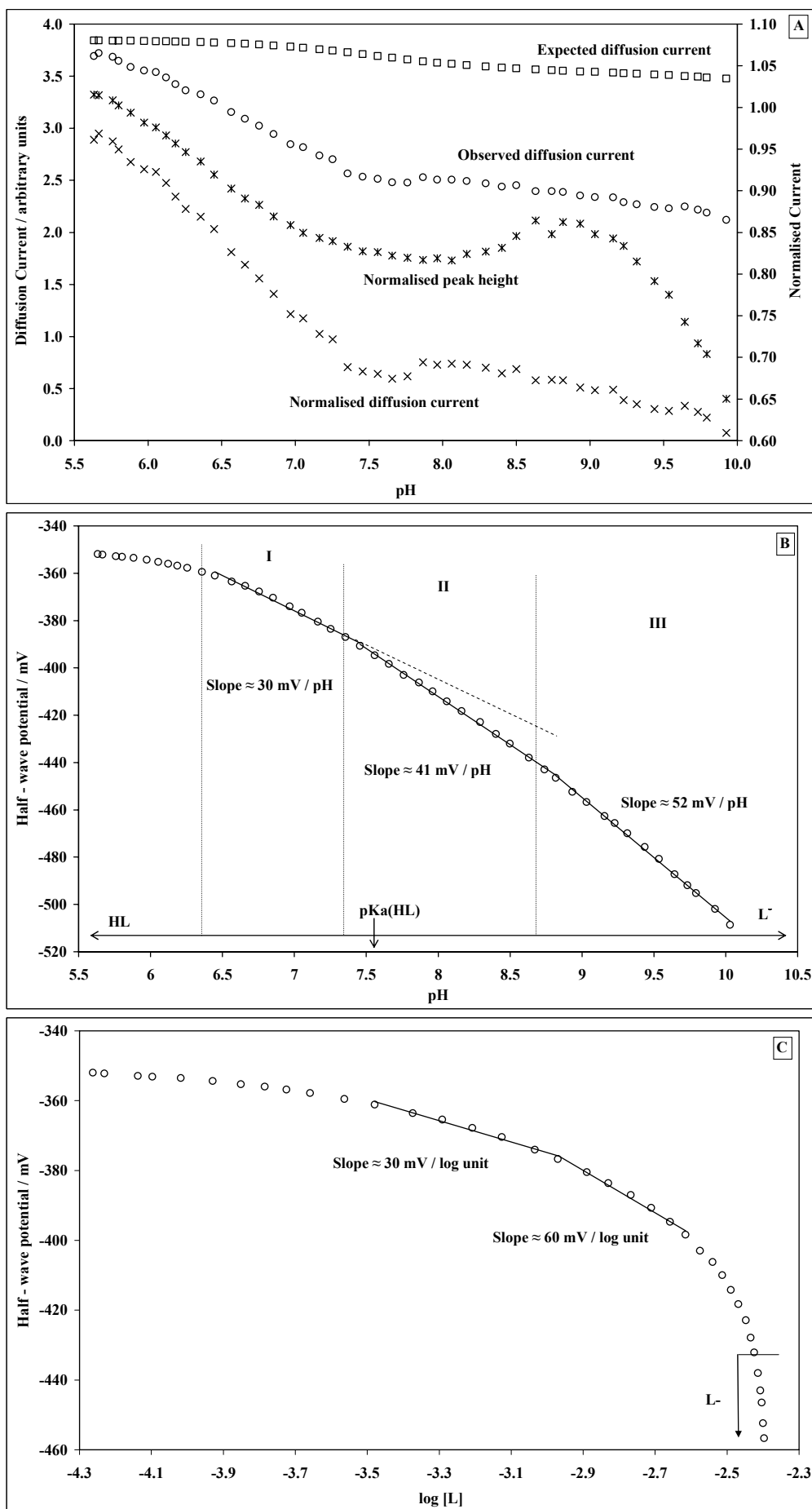


Figure 5-2 Analysis of the DPP (only A) and DCP (A, B and C) data for $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system. DCP → and DPP results were obtained with the same solution for $[\text{Pb}^{2+}] = 4.5 \times 10^{-5} \text{ M}$, $[\text{L}_T]:[\text{Pb}_T] = 102$, 0.1 M KNO_3 at 25.0 °C. (A) Variation in the intensity of DCP waves throughout the polarographic titration. Circles, squares and cross represent the observed, expected if no complexes were formed and corrected for dilution normalised currents, respectively. Asterisks represent the variation of the normalised peak height for DPP experiment. (B) Half-wave potential as a function of pH; Dashed line represents a theoretical slope of 29.5mV that should be obtained if there was an involvement of one proton in the electrochemical reaction. (C) Half-wave potential as a function of the free ligand concentration.



The analysis of the graph $E_{1/2}$ vs. $\log [L]$ (Figure 5-2C) was used to confirm the formation of PbL_n species. From $\log [L]$ -3.5 to -3.0 (this is equivalent to the pH range between 6.4 and 7.0, first region in Figure 5-2C), a slope of about 30 mV was recorded, which evidences the formation of PbL species, as predominant, in this pH range. At larger free ligand concentration, a slope around 60 mV was detected, indicating the formation of PbL_2 species (between pH 7.0 and 7.7 in Figure 5-2C).

From the graphic analysis described above, the proposed model for $Pb-(TAPSO)_x-(OH)_y$ system should incorporate the following species: PbL , PbL_2 , $PbL_2(OH)$ and $PbL_2(OH)_2$ and possibly $PbL(OH)$, $PbL(OH)_2$, the later ones most likely as minor species.

5.3.3 MODELLING PROCEDURES AND REFINEMENT OF STABILITY CONSTANTS

5.3.3.1 POLAROGRAPHY

The optimisation of the model and the refinement of the stability constants were performed using the experimental points and calculating the complex formation curves (Figure 5-3A). This iterative process was finished when the CCFC fitted best the ECFC, which is indicated by the standard deviation of the fitting (SD) between the ECFC and the CCFC. In Figure 5-3A, the large difference between ECFC (circles) and CCFC for the $Pb_x(OH)_y$ species alone (curve 1) clearly evidences that complexes $PbL_x(OH)_y$ are formed throughout all pH-range indicated. This figure also shows that in the absence of TAPSO complexation, precipitation of $Pb(OH)_2$ is predicted at $pH \approx 8.3$, which was not observed. The strategy adopted in the refinement of stability constants using polarographic data is presented in Table 5-2.

Table 5-2 Overall stability constants for $Pb-(TAPSO)_x-(OH)_y$ system determined at 25 °C and $I = 0.1$ M by DCP for $[L_T]:[Pb_T] = 102$, $[Pb^{2+}] = 4.5 \times 10^{-5}$ M.

Complexes	Equilibrium	Model 1	Model 2
PbL^+	$Pb^{2+} + L^- \rightleftharpoons PbL^+$	3.60 ± 0.02	3.63 ± 0.03
$PbL(OH)$	$Pb^{2+} + L^- + OH^- \rightleftharpoons PbL(OH)$	10.44 ± 0.01	NI
$PbL(OH)_2^-$	$Pb^{2+} + L^- + 2OH^- \rightleftharpoons PbL(OH)_2^-$	15.46 ± 0.01	NI
PbL_2	$Pb^{2+} + 2L^- \rightleftharpoons PbL_2$	R	6.48 ± 0.06
$PbL_2(OH)^-$	$Pb^{2+} + 2L^- + OH^- \rightleftharpoons PbL_2(OH)^-$	NI	12.88 ± 0.02
$PbL_2(OH)_2^{2-}$	$Pb^{2+} + 2L^- + 2OH^- \rightleftharpoons PbL_2(OH)_2^{2-}$	NI	17.84 ± 0.01
SD / mV		0.23	0.17

SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (see Part II, Chapter 2, Section 2.3.3). NI – Not included; R – Rejected

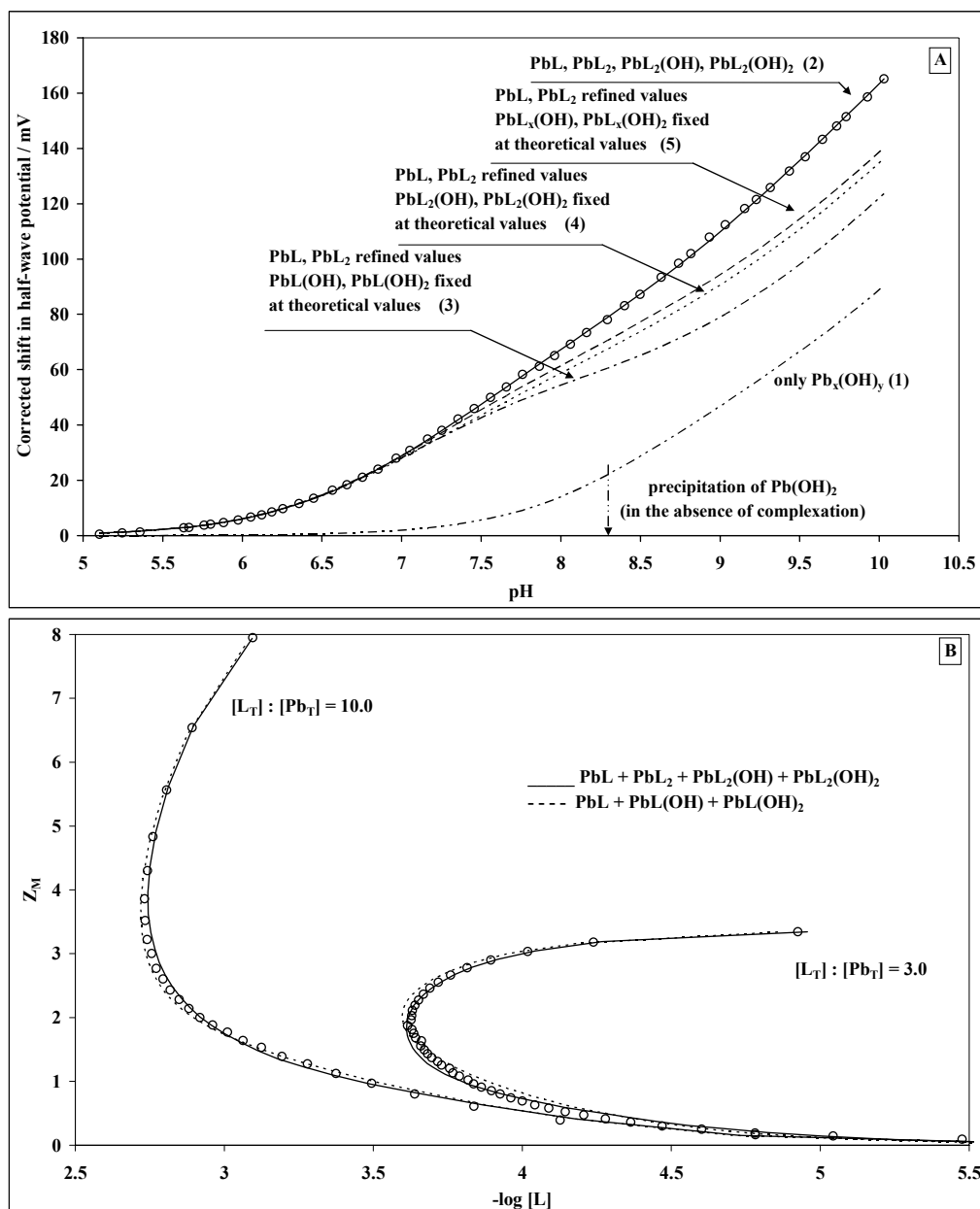


Figure 5-3 Experimental (circles) and calculated (lines) complex formation curves obtained for the models indicated in the figure for DCP (A) and GEP (B) data for $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system. (A) $[\text{L}_T]:[\text{Pb}_T] = 102$, $[\text{Pb}^{2+}] = 4.5 \times 10^{-5}$ M. Solid line going through the experimental points (curve 2) was obtained for the optimised model and refined stability constants (Model 2, Table 5-2). Refined stability constants used for PbL and PbL_2 were (as $\log \beta$) 3.63 and 6.48, respectively. (B) $[\text{Pb}^{2+}] = 5.2 \times 10^{-4}$ M, $[\text{L}_T]:[\text{Pb}_T] = 3.3$, and $[\text{Pb}^{2+}] = 4.8 \times 10^{-4}$ M, $[\text{L}_T]:[\text{Pb}_T] = 10.0$, at 25.0 °C and 0.1 M KNO_3 . Values of the stability constants used for models were those described for Models 1 and 2 in Table 5-3 for each $[\text{L}_T]:[\text{Pb}_T]$ ratio.

We started by two simplified models: PbL, PbL₂, PbL(OH) and PbL(OH)₂ (Model 1) and PbL, PbL₂, PbL₂(OH) and PbL₂(OH)₂ (Model 2). The refinement of Model 1 resulted in the rejection of PbL₂. After that, the reduced in size model, PbL, PbL(OH) and PbL(OH)₂ was fitted without a problem (Model 1, Table 5-2). Nevertheless, this model does not describe the system as we have clear evidence of the presence of PbL₂ in solution (Figure 5-2C). Simplified model PbL, PbL₂, PbL₂(OH) and PbL₂(OH)₂ (Model 2, Table 5-2) was also fitted without a problem with almost the same, but slightly better, standard deviation in the overall fit. The CCFC for this model is illustrated in Figure 5-3A, curve 2, which describes very well the experimental data.

The values obtained for the stability constants for the hydroxide species, PbL_x(OH)_y, are a bit larger than the expected ones. For PbL(OH), the theoretical value would be 9.63 ($\log \beta_{\text{PbL(OH)}} = \log \beta_{\text{Pb(OH)}} + \log \beta_{\text{PbL}} \Leftrightarrow \log \beta_{\text{PbL(OH)}} = 6.0 + 3.63$) and for PbL(OH)₂ 13.93 ($\log \beta_{\text{PbL(OH)}_2} = \log \beta_{\text{Pb(OH)}_2} + \log \beta_{\text{PbL}} \Leftrightarrow \log \beta_{\text{PbL(OH)}_2} = 10.3 + 3.63$), while the ones that were obtained are larger (Model 1, Table 5-2). Using the same procedure, the theoretical values for PbL₂(OH)_x complexes are: $\log \beta_{\text{PbL}_2\text{(OH)}} = \log \beta_{\text{Pb(OH)}} + \log \beta_{\text{PbL}_2} \Leftrightarrow \log \beta_{\text{PbL}_2\text{(OH)}} = 6.0 + 6.48 = 12.48$, $\log \beta_{\text{PbL}_2\text{(OH)}_2} = \log \beta_{\text{Pb(OH)}_2} + \log \beta_{\text{PbL}_2} \Leftrightarrow \log \beta_{\text{PbL}_2\text{(OH)}_2} = 10.3 + 6.48 = 16.78$. Using these theoretical values, three CCFC were generated (Figure 5-3A, curves 3 to 5). Analysis of CCFC for models described by curves 3 and 4 in Figure 5-3A, with PbL_x(OH)_y species fixed at theoretical values, suggests that PbL₂(OH)_y species are major species at higher pH values; curve 4 is much closer to the ECFC than curve 3, which means that PbL(OH)_y species exist in a small amount. This is also confirmed when curves 4 and 5 are compared. Including all the hydroxide species, fixed at theoretical values (curve 5, Figure 5-3A), the difference between curves 4 and 5 is minimal, evidencing that the contribution of PbL(OH)_y species is of about 4 mV.

Taking into account all that was described above, the solid line (curve 2) represents the major species present in solution, with refined stability constant values indicated in Table 5-2. From a statistical point of view, no more species are required to reproduce the objective function. The generated SDD for the DCP experimental conditions (Figure 5-4A) agrees satisfactorily with the DCP data.

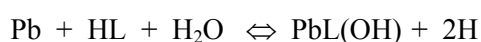
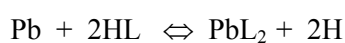
For example, PbL is a major species in solution between pH 6.4 and 7.4 and above pH 8.7 the main species in solution is PbL₂(OH)₂, as was predicted by slopes analysis (Figure 5-2B, C). The SDD also evidences the formation of PbL₂ in the pH range between 7.0 and 7.7, as a non predominant species, which does not explain well the slope of 60 mV obtained in the $E_{1/2}$ vs. $\log [L]$ plot (Figure 5-2C). This is an indication that the stability constant for this complex should be a little bit higher.

5.3.3.2 POTENTIOMETRY

Since polarographic studies provided data at relatively large [L_T]:[Pb_T] ratios, it was expected that additional experiments using GEP, which works at much lower ratios, might provide significant contribution to the model and stability constants. Strategies in the refinement were similar to those used for DCP data and are summarised in Table 5-3. For [L_T]:[Pb_T] = 3.3, PbL₂ was rejected in Model 1 (as occurred in polarography) and the simplified Models 1 and 2 resulted in stability constants with smaller standard deviations for all species (Table 5-3, Part A). Here again, the simplified Model 2 was slightly favoured by refinement operations. The refinement of acid concentration resulted in lower values for all stability constants (unless for PbL₂, Model 2 and 2') and smaller standard deviations, with a variation in acid concentration of about 2%. This emphasises how demanding is GEP in terms of the quality of experimental data. But still all the stability constants values seemed to be too large, particularly PbL₂(OH)_y. This can be explained by the small amount of data points that were available for the determination of these constants. It appears that this ratio was too low. It has been decided to investigate the system at another ratio, higher than commonly used for potentiometry, at [L_T]:[Pb_T] = 10.0 (Table 5-3, Part B). The behaviour was similar to the other GEP ratio, but the stability constant values were lower than those from ratio 3.3 and much closer to polarographic values. In this case, the refinement of acid concentration did not change the results (data not shown).

Values of the complex formation function, Z_M defined elsewhere (Part II, Chapter 2, Section 2.4.3), were calculated for each datum point (for [L_T]:[Pb_T] ratio of 3.3 and 10.0) to aid in the modelling procedures (see Figure 5-3B). For [L_T]:[Pb_T] ratio of 10.0, the analysis of Figure 5-3B shows that Z_M function increases linearly until 1.0, indicating that there is PbL in the solution, then continues to grow until 2.0, but with a lower slope, indicating the presence of PbL₂ in small amount. Then, the function starts to curve back, indicating that there is formation of hydroxide species in the solution. On the other hand, for [L_T]:[Pb_T] ratio of 3.3, although it looks like that there is no PbL₂, it seems that model PbL, PbL₂, PbL₂(OH) and PbL₂(OH)₂ (Model 2, Table 5-3, Part A) fits slightly better the experimental data than model PbL, PbL(OH) and PbL(OH)₂, (Model 1, Table 5-3, Part A), as it is also demonstrated by the R-factor.

Incorporating all possible lead species for [L_T]:[Pb_T] ratio 10.0, it resulted in the rejection of PbL(OH) and of PbL₂(OH)₂ (Model 3, Table 5-3). The rejection of PbL(OH) can be explained by the fact that the same amount of protons is involved in the formation of PbL₂ or PbL(OH):



which makes it difficult for GEP to distinguish them easily and decide on any of them.

Table 5-3 Refinement strategy for GEP titrations. Overall stability constants for $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system determined at 25 °C and $\mu = 0.1 \text{ M}$ by GEP for (Part A) $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 3.3$, $[\text{Pb}^{2+}]:[\text{Pb}_\text{T}] = 5.2 \times 10^{-4} \text{ M}$, two titrations, 95 points, between pH 3.9 and 8.0 and (Part B) $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 10.0$, $[\text{Pb}^{2+}] = 4.8 \times 10^{-4} \text{ M}$, three titrations, 137 points, between pH 3.5 and 11.0.

Complexes	Equilibrium	Part A. [L _T] : [Pb _T] = 3.3				Part B. [L _T] : [Pb _T] = 10.0			
		Model 1	Model 1'	Model 2	Model 2'	Model 1	Model 2	Model 3	Model 4
PbL ⁺	Pb ²⁺ + L ⁻ ⇌ PbL ⁺	4.13±0.02	3.793±0.006	4.20±0.02	3.80±0.01	3.90±0.02	3.93±0.02	3.93±0.02	3.93±0.02
PbL(OH)	Pb ²⁺ + L ⁻ + OH ⁻ ⇌ PbL(OH)	10.85±0.03	10.682±0.005	NI	NI	10.76±0.03	NI	R	9.93 (F)
PbL(OH) ₂ ⁻	Pb ²⁺ + L ⁻ + 2OH ⁻ ⇌ PbL(OH) ₂ ⁻	16.31±0.06	15.71±0.02	NI	NI	15.54±0.04	NI	15.58±0.04	14.23 (F)
PbL ₂	Pb ²⁺ + 2L ⁻ ⇌ PbL ₂	R	R	6.8±0.2	7.27±0.01	NI	6.76±0.06	6.76±0.06	6.65±0.07
PbL ₂ (OH) ⁻	Pb ²⁺ + 2L ⁻ + OH ⁻ ⇌ PbL ₂ (OH) ⁻	NI	NI	14.16±0.03	13.80±0.01	NI	13.26±0.04	13.25±0.04	13.21±0.05
PbL ₂ (OH) ₂ ²⁻	Pb ²⁺ + 2L ⁻ + 2OH ⁻ ⇌ PbL ₂ (OH) ₂ ²⁻	NI	NI	R	18.31±0.06	NI	17.99±0.04	R	17.98±0.04
Hamilton R-factor		0.0665	0.0105	0.0605	0.0096	0.0150	0.0146	0.0144	0.0146
[H ⁺] (M)		0.001840	0.001882*	0.001840	0.001881*				

Hamilton R-factor stands for a statistical parameter generated by the program ESTA (see Part V, Chapter 10, Section 10.2.4.1). * refined value.

Similar situation occurs at higher pH values, where the ligand is fully deprotonated, between $\text{PbL}(\text{OH})_2$ and $\text{PbL}_2(\text{OH})_2$.

The incorporation of $\text{PbL}(\text{OH})$ and $\text{PbL}(\text{OH})_2$ in Model 2, at theoretical fixed values, did neither modify significantly the stability constants values nor improve the Hamilton R-factor – see Model 4 for $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ equal to 10.

Generating species distribution diagrams for GEP conditions (Table 5-3, Part B, Model 4) (Figure 5-4B), which corresponds to lower ratios with higher metal concentration and thus would favour $\text{PbL}(\text{OH})_y$ species, the amount of $\text{PbL}(\text{OH})$ and $\text{PbL}(\text{OH})_2$ formed is very small. This is an additional evidence that the final model should only include PbL , PbL_2 , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$, with PbL_2 as a major but not predominant metal containing species (Figure 5-4B).

5.3.4 OVERALL STABILITY CONSTANT VALUES

Combining the results obtained by both electrochemical techniques (DCP and GEP) and using graphic analysis (CCFC versus ECFC for DCP and Z_M function for GEP) for both techniques, as well as species distribution diagrams, a graphical iterative process was performed that resulted in the final $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ model and stability constants (as $\log \beta$) as: $\text{PbL} = 3.7 \pm 0.1$, $\text{PbL}_2 = 6.6 \pm 0.1$, $\text{PbL}_2(\text{OH}) = 12.9 \pm 0.1$, and $\text{PbL}_2(\text{OH})_2 = 17.8 \pm 0.1$. Note that the standard deviations indicate here the ranges in stability constants obtained from different ratios and techniques and are within the experimental errors associated with all techniques.

To test the proposed model, species distribution diagrams were generated for $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratio 3.3, used in GEP experimental conditions, and ratio 102, used in DCP experimental conditions (Figure 5-5).

Figure 5-5A suggests that PbL and $\text{PbL}_2(\text{OH})$ are the major species present in solution before precipitation occurs, which is in agreement with GEP results, where the fit model PbL , PbL_2 and $\text{PbL}_2(\text{OH})$ resulted in a better R-factor (Models 2 and 2', Table 5-3 Part A). In addition, SDD predicts precipitation around pH 8.0, explaining the difficulty of obtaining a reliable value for $\log \beta_{\text{PbL}_2(\text{OH})_y}$. It is also possible to see the beginning of formation of polynuclear metal hydroxide species, which does not favour the refinement procedures. From SDD, it is clear that the main species present in solution and the one that it is possible to refine with some accuracy is PbL as it was shown by the results (Table 5-3). Also for the $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratio 102, SDD suggests that PbL , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$ are the major species present in solution (Figure 5-5B), and this was exactly established from the DCP experiments.

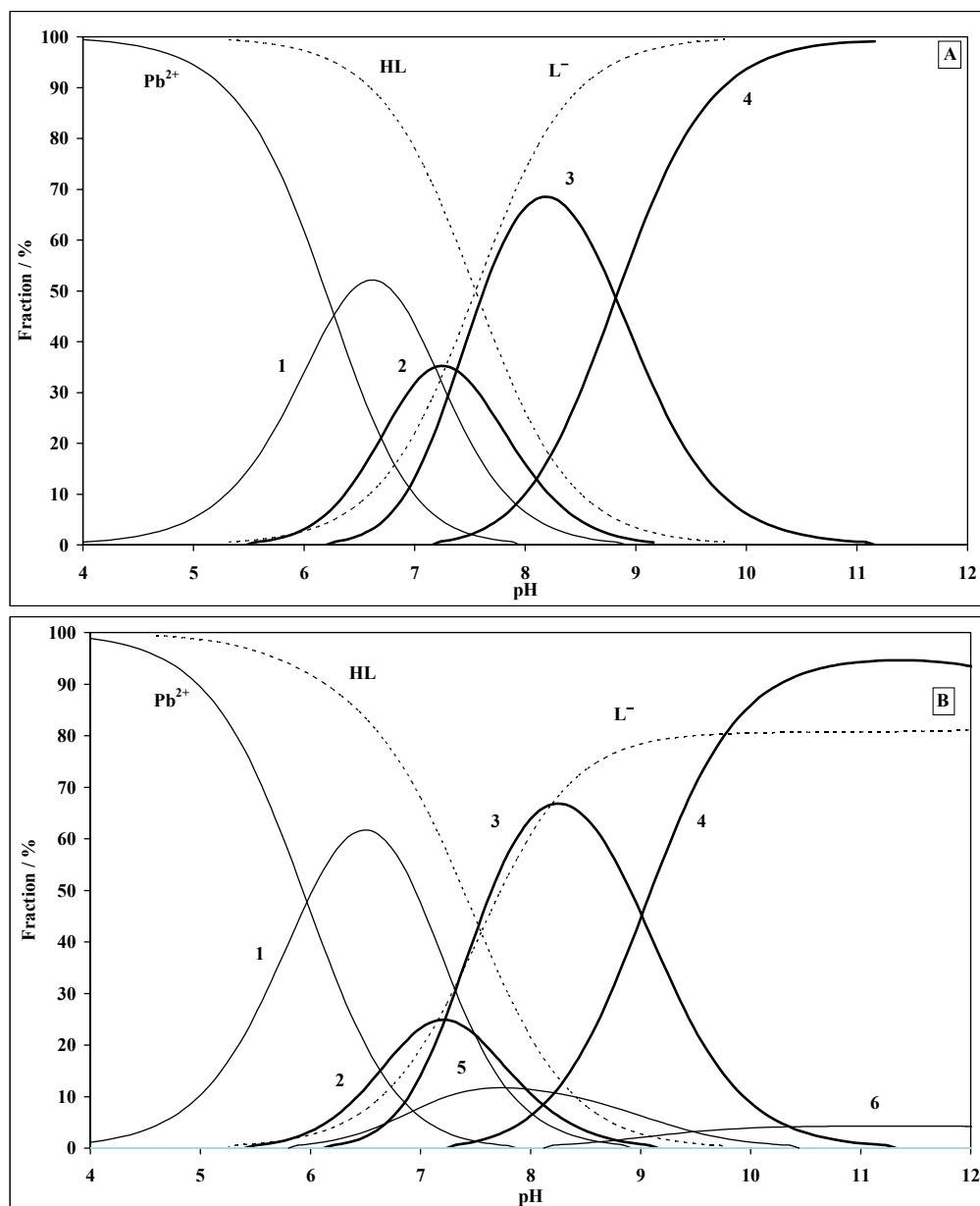


Figure 5-4 Species distribution diagrams as a function of pH calculated for the Pb-(TAPSO)_x-(OH)_y system based on DCP (A) and GEP (B) results reported in this work at 25.0 °C and 0.1 M KNO₃. (A) [Pb²⁺] = 4.5×10^{-5} M, [L_T]:[Pb_T] = 102. The metal-ligand model consisted of PbL (1), PbL₂ (2), PbL₂(OH) (3), and PbL₂(OH)₂ (4) for which stability constants, as log β, were set to 3.63, 6.48, 12.88, and 17.84, respectively. (B) [Pb²⁺] = 4.8×10^{-4} M, [L_T]:[Pb_T] = 10.0. The plotted metal-ligand model is Model 4 (Table 5-3 Part B). PbL (1), PbL₂ (2), PbL₂(OH) (3), PbL₂(OH)₂ (4), PbL(OH) (5) and PbL(OH)₂ (6), for which stability constants, as log β were 3.93, 6.65, 13.21, 17.98, 9.93, 14.23, respectively.

The comparison of $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ and $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$ (Part II, Chapter 2) systems shows that copper binds stronger with TAPSO than lead; a normal tendency behaviour well described in the literature [8]. This fact resulted in different models for both systems; for copper, a more complex model was obtained where six species were defined: CuL , $\text{CuL}(\text{OH})$, $\text{CuL}(\text{OH})_2$, CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$, with stability constants, as $\log \beta$, 4.41, 11.43, 17.55, 8.08, 14.3 and 20.3, respectively (Part II, Chapter 2).

Comparing both models, it raises that $\text{PbL}(\text{OH})_y$ species were not evidenced for $\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y$ system. Because $\log \beta_{\text{PbL}_2}$ is about 1.1 times smaller than $\log \beta_{\text{CuL}_2}$, the formation of PbL_2 complex occurs at higher pH values than CuL_2 . PbL_2 is mainly formed near the pK_a of TAPSO (the maximum percentage of complex occurs around pH 7.2, Figure 5-5B), while CuL_2 is formed 0.5 pH units below (Part II, Chapter 2). This fact associated to the lower stability constants expected for $\text{PbL}(\text{OH})$ species, if this species was formed, explains that even for lower $[\text{L}_T]:[\text{Pb}_T]$ ratios, PbL_2 is favoured instead of $\text{PbL}(\text{OH})$ at pH 7.5 - 7.8 (Figure 5-4B) because a large amount of ligand is deprotonated.

In conclusion, the results obtained have evidenced the formations of several complex species, PbL , PbL_2 , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$. Three of these complexes, PbL , PbL_2 and $\text{PbL}_2(\text{OH})$, are mainly formed in the buffer pH range. These results evidence that the presence of these complexes should not be neglected when TAPSO is used, as a buffer, in studies involving lead ion. Because the concentration of buffer is usually quite high, between 10^{-3} and 0.1 M, this can generate significant differences in the free lead ion concentration.

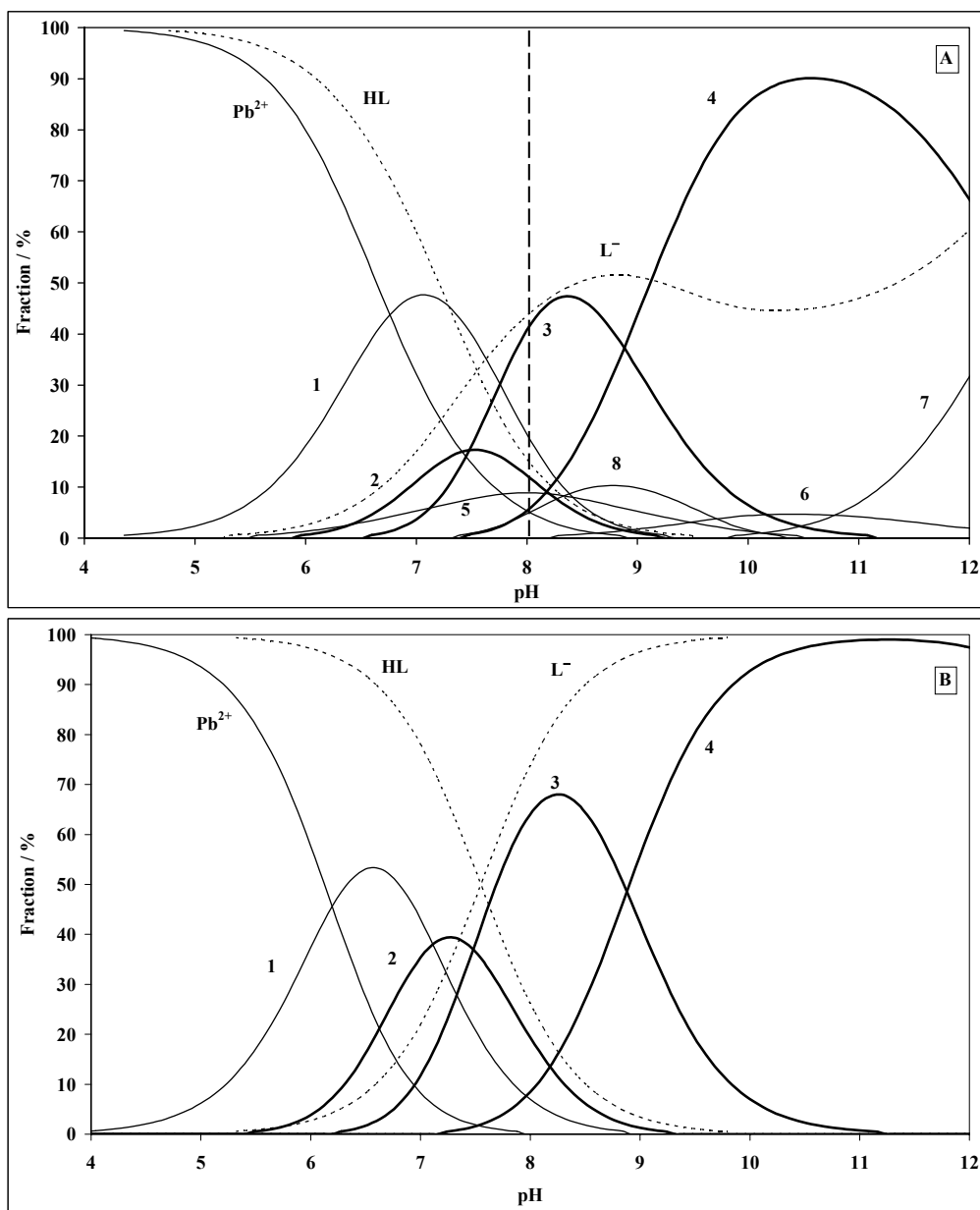


Figure 5-5 Species distribution diagrams as a function of pH computed for Pb-(TAPSO)_x-(OH)_y system. [Pb²⁺] = 5.2 × 10⁻⁴ M, [L_T]:[Pb_T] = 3.3 (A) and [Pb²⁺] = 4.5 × 10⁻⁵ M, [L_T]:[Pb_T] = 102 (B). The metal-ligand model consisted of PbL (1), PbL₂ (2), PbL₂(OH) (3), and PbL₂(OH)₂ (4) for which stability constants, as log β, were set to 3.7, 6.6, 12.9, and 17.8, respectively. Values of Pb(OH) (5), Pb(OH)₂ (6), Pb(OH)₃ (7) and Pb₃(OH)₄ (8) stability constants are in Table 5-1. The vertical dashed line in part A represents precipitation of Pb(OH)₂ at pH = 8.0.

6 MODELLING OF Pb-(TAPS)_x-(OH)_y SYSTEM AND REFINEMENT OF STABILITY CONSTANTS, IN THE REGION OF LEAD HYDROLYSIS AND LEAD HYDROXIDE PRECIPITATION, BY POLAROGRAPHIC AND POTENTIOMETRIC OPTIMISATION TOOLS: IMPORTANCE OF A MULTI-TECHNIQUE APPROACH⁵

ABSTRACT

The influence of TAPS on solutions containing lead(II) was studied by GEP and DCP. The readings were taken at fixed total TAPS to total lead(II) concentration ratios and various pH values, at 25.0 ± 0.1 °C and ionic strength 0.1 M KNO₃. Due to the basic pK_a of the ligand, which occurs in the pH range where large amount of lead polynuclear species are formed, and the occurrence of ligand adsorption, that disabled the use of high concentrations of TAPS on DCP experiments, neither GEP nor DCP were able to provide, when used independently, the correct Pb-(TAPS)_x-(OH)_y model and reliable stability constants. Final model was defined after an exhaustive comparative analysis performed between the species distribution diagrams generated for DCP and GEP experimental conditions used and the refined data. The proposed final model is: PbL, PbL₂(OH) and PbL₂(OH)₂ with overall stability constants values, as $\log \beta$, 3.29 ± 0.04 , 12.1 ± 0.1 , and 17.0 ± 0.1 , respectively. A comparative analysis of the strength of complexation of TAPS and a structural related buffer, TAPSO, with lead is also discussed.

6.1 INTRODUCTION

Hydrogen ion concentration plays an important role in the chemistry of aqueous solutions because it competes with metallic cations in solution for ligand binding sites. Therefore, metal ion speciation studies are usually carried out at a constant pH to simplify the interpretation of the results. For this purpose, it is frequently necessary to add a pH buffer. However, the choice of a pH buffer may constitute an important problem since many of them (inorganic or organic ones) traditionally used are also complexing agents and may disturb the equilibrium state. For example, TRIS is a pH buffer currently used for speciation studies [13-16], even though it complexes metal ions [8].

The biological buffer TAPS is chemically related with TRIS and has a pK_{a2} value that allows buffering the pH in the range between 7.3 and 9.3, which covers the range of pH that occurs in the environmental systems. So, TAPS can be an alternative choice when heavy metal speciation studies

⁵ Submitted for publication

are undertaken.

TAPS is now commercially available at least by two major chemical suppliers [17, 18]. However, because TAPS contains hydroxyl groups in addition to amine nitrogen, as it happens with TRIS, metal complexes of TAPS is expected to be formed in environmental systems containing metal ions when this compound is used to control the pH.

As far as we know, no reports regarding studies of the interaction between TAPS and lead are described in the literature. Thus, we think that the knowledge of the stability constants for Pb–(TAPS)_x–(OH)_y system will upgrade the information about the stability constants of ions complexation with biological buffers commercially available.

In the present work, we report the overall stability constants for the species formed between TAPS and lead ion, which is an important environmental metal ion, at 0.1 M KNO₃ ionic strength and 25.0 ± 0.1°C. The characterization of the Pb–(TAPS)_x–(OH)_y system was done by DCP and GEP using total ligand to total metal concentration ([L_T]:[Pb_T]) ratios of various orders of magnitude. Experiments by ACP were also carried out to check if TAPS adsorbs at the mercury working electrode surface, under the experimental conditions used.

6.2 EXPERIMENTAL

6.2.1 MATERIALS AND REAGENTS

The standard lead solution, 9.6×10^{-3} M, was purchased from Merck.

Additional information about the remaining materials and reagents were described previously (Part II: Chapter 2, Section 2.2.1 and Chapter 4, Section 4.2.1).

6.2.2 APPARATUS

All experiments were carried out in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer, and thermostatted at 25.0 ± 0.1 °C using a water bath.

6.2.2.1 POLAROGRAPHY

Polarographic measurements, for Pb-(TAPS)_x-(OH)_y system, were performed as previously described (Part II, Chapter 4, Section 4.2.2.1).

6.2.2.2 POTENTIOMETRY

The potentiometric experiments reported in this work, were performed as previously described (Part II, Chapter 2, Section 2.2.2.2).

6.2.3 PROCEDURE

6.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

For both techniques used, the calibration of the glass electrode (pH measured as $-\log [H^+]$) was accomplished as previously described (Part II, Chapter 3, Section 3.2.3.1).

6.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

In order to evaluate the adsorption of the ligand TAPS on the surface of the mercury of the working electrode, ACP polarograms were run in the absence of the metal ion. The experimental conditions used were described elsewhere (Part II, Chapter 4, Section 4.2.3.2).

6.2.3.3 DIRECT CURRENT POLAROGRAPHY

The study of the electrochemical behaviour of Pb-(TAPS)_x-(OH)_y system was performed by DCP. A drop time of 1s and a step potential of 4 mV were used. The procedure used to run the polarographic titrations was described previously (Part II, Chapter 2, Section 2.2.3.3). Two [L_T]:[Pb_T] ratios were used, 88.7 and 124.3, with [Pb²⁺] of 9.6×10^{-6} M and 2.4×10^{-5} M, respectively. The scanned pH range varied between 6.4 and 10.0. About 60 points were obtained for each ratio. The equilibrium of the metal-ligand solutions was tested and it was reached in a few minutes.

6.2.3.4 GLASS ELECTRODE POTENTIOMETRY

For studying potentiometrically the $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system, two different $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratios were performed: $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 6.2$ with $[\text{Pb}^{2+}] = 9.7 \times 10^{-4} \text{ M}$ (five titrations were performed using two independent solutions; for each titration, about 70 points were collected between pH 5.0 and 9.5) and $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 10.4$, $[\text{Pb}^{2+}] = 9.7 \times 10^{-4} \text{ M}$ (two titrations were performed and, for each titration, about 240 points were collected between pH 4.9 and 9.0). Monotonic additions of standardized KOH 0.1M were done.

6.2.4 DATA TREATMENT

The refinement of polarographic data was done using the Cukrowski method [4, 5]; as it was described previously (Part II, Chapter 2, Section 2.2.4).

The simulation and optimization of model and complex formation constants from potentiometric titrations data were performed using ESTA program [6, 7]; for more details about the program see Part II, Chapter 2, Section 2.3.3 and Part V, Chapter 10, Section 10.2.4.1.

During the refinement operations of the different systems, the water [8] (Part II, Chapter 2, Table 2-1) and ligand (Part II, Chapter 4, Table 4-1) protonation constants, as well as all known formation constants for lead hydroxide species, $\text{Pb}_x(\text{OH})_y$ [8] (Part III, Chapter 5, Table 5-1), were kept fixed.

6.3 RESULTS AND DISCUSSION

6.3.1 TAPS ADSORPTION AND LEAD REVERSIBILITY STUDIES

Metal stability constants calculated by polarographic techniques can be significantly influenced by the ligand and/or complexes adsorption, the best experimental conditions would be in the absence of adsorption of the ligand/complexes. Therefore, ACP at a phase angle of 90° was used to test if there was adsorption of TAPS at the mercury electrode surface. The adsorption effect can be quantified by comparing the AC polarograms for the background solution with the polarograms obtained with increasing ligand additions. Figure 6-1 shows the variation in the capacitive current (expressed in percentage) obtained with the increase of ligand concentration relatively to the background solution (0.1 M KNO_3), at a reduction potential typical for Pb^{2+} (-0.35V [11]), for three pH values, pH 6.34, 8.34 and 10.34. For pH = 6.34, where the ligand is totally in the HL form, the variation in the capacitive current with the increase of the ligand concentration was less than 5% for all the TAPS

concentration range tested. At $\text{pH} = 10.34$, the variation in the capacitive current was slightly pronounced than for other pH values, being recorded a variation of 19% for the highest TAPS concentration tested, $1.2 \times 10^{-2} \text{ M}$; for $3.0 \times 10^{-3} \text{ M}$ of TAPS, about 9% variation in the capacitive current was observed. This increase of the adsorption effect in the region of zero charge potential (about -0.4 V), i.e., the region where the surface of mercury electrode has no charge, suggests that, at this potential, the adsorption of TAPS is mainly due to the uncharged part of the ligand. For $\text{pH} = \text{pK}_a$ (where the ligand exists half in the protonated form, HL , and half in the deprotonated form, L^-), a midway situation between the other ones was recorded (Figure 6-1). The results described above point out that a maximum TAPS concentration of $3.0 \times 10^{-3} \text{ M}$ should be used for studying $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system by DCP.

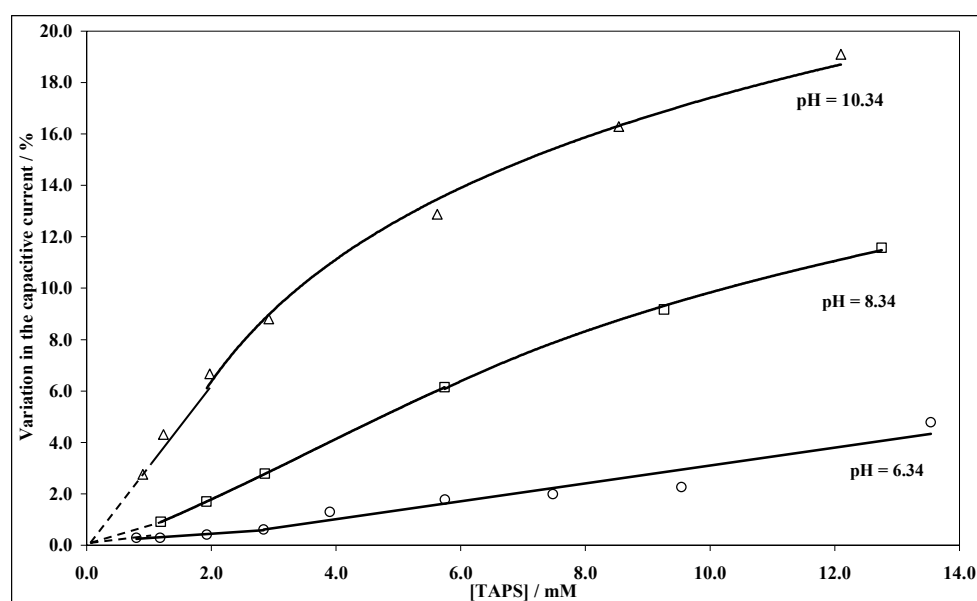


Figure 6-1 Variation in the capacitive current recorded by ACP with the increase of TAPS concentration at three pH values, 6.34, 8.34 and 10.34. The values, expressed in percentage, were calculated from the difference between the capacitive current recorded in the presence and in the absence of the buffer, at -0.35 V , from ACP curves with a phase angle of 90° in 0.1 M KNO_3 .

The analysis of polarographic titrations for $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system showed that the addition of the ligand caused a decrease in the recorded DC wave of the metal of about 20 % and the position of the polarographic signal has changed by about 3 mV towards more positive values for both ratios. This could be due to some minor adsorption effect. To correct for that phenomenon, the half-wave potential and the diffusion current of the metal ion in the presence of the ligand (values recorded in a pH region where no complexation occurs) was used, as a reference point, for computing the shift in the peak potential. In addition, the wave steepness values did not change significantly (values varied between 0.9 and 1.1) for both titrations performed, indicating that lead reduction process at the mercury electrode surface was fully reversible.

6.3.2 DCP GRAPHICAL PREDICTION OF SPECIES PRESENT IN SOLUTION

The analysis of DC polarographic data in all the pH range tested showed that $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system behaved as labile and dynamic on the polarographic time scale; only one peak corresponding to all labile species present in solution, which shifted to more cathodic potential values, was observed (data not shown).

For DCP data, species prediction present in solution was accomplished by graphical analysis and interpretation of the slopes of the variation of the observed half-wave potential shift as a function of the pH (Figure 6-2A) and of the logarithm of the free ligand concentration (Figure 6-2B). As an example, the results obtained for $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 88.7$ will be analyzed below. Similar results were obtained for the other ratio (data not shown). From Figure 6-2A, four regions corresponding to different slopes were identified. The value of the protonation constant and the respective forms of the ligand were also marked in the figure. The possible reactions at the DME, according to the pH of the solution, are also marked in the figure (charges were omitted for sake of simplicity). The first slope, drawn between pH 7.5 and 8.1, has a value of 12 mV / pH. This value can indicate some interaction between lead and TAPS, as PbL species. However when one proton is involved in the reaction at the DME electrode (formation of PbL) a theoretical slope of 29.5 mV / pH should be obtained, as it is indicated in Figure 6-2A. Additionally, the reduction of $\text{Pb}(\text{OH})$ species also develops a slope of similar magnitude (Figure 6-2A). The fact that the observed slope did not develop to the theoretical value of 29.5 mV / pH can be related to the form of the ligand present in solution. Above pH 7.34 already some amount of deprotonated ligand is present in solution and in this case the reduction of PbL at the DME electrode does not involve protons, $\text{PbL} + 2\text{e} \rightleftharpoons \text{Pb}(\text{Hg}) + \text{L}$, resulting in a slope equal to 0 mV/pH, which can explain why the slope between 7.5 and 8.1 does not develop to its theoretical value. Between pH 8.1 and 8.6, a slope of 31 mV / pH was recorded (Figure 6-2A). That is exactly the region of the ligand deprotonation, which means that the two forms of the ligand, protonated and deprotonated, are present in solution. As it was mentioned above, the formation of PbL would result in a slope of 29.5 mV / pH if there was the involvement of one proton in the reaction at the DME electrode. However, the formation of this species above the pK_a , where the main form of the ligand is the deprotonated one, would result in even larger decrease of the slope since the reduction reaction does not involve any proton, as mentioned before. So, the formation of PbL , as a major species in this pH region, does not seem probable. The observed slope, 31 mV/pH, can be explained by the reduction of $\text{Pb}(\text{OH})$ species (Figure 6-2A). The formation of $\text{PbL}_x(\text{OH})$ species ($x = 1$ and/or 2), before deprotonation, would result in a theoretical slope higher than 29.5 mV / pH, which would increase the slope. Above the pK_a , a theoretical slope of 29.5 mV / pH would be generated. Thus, the formation of some amount of these species, in this pH range, cannot be excluded.

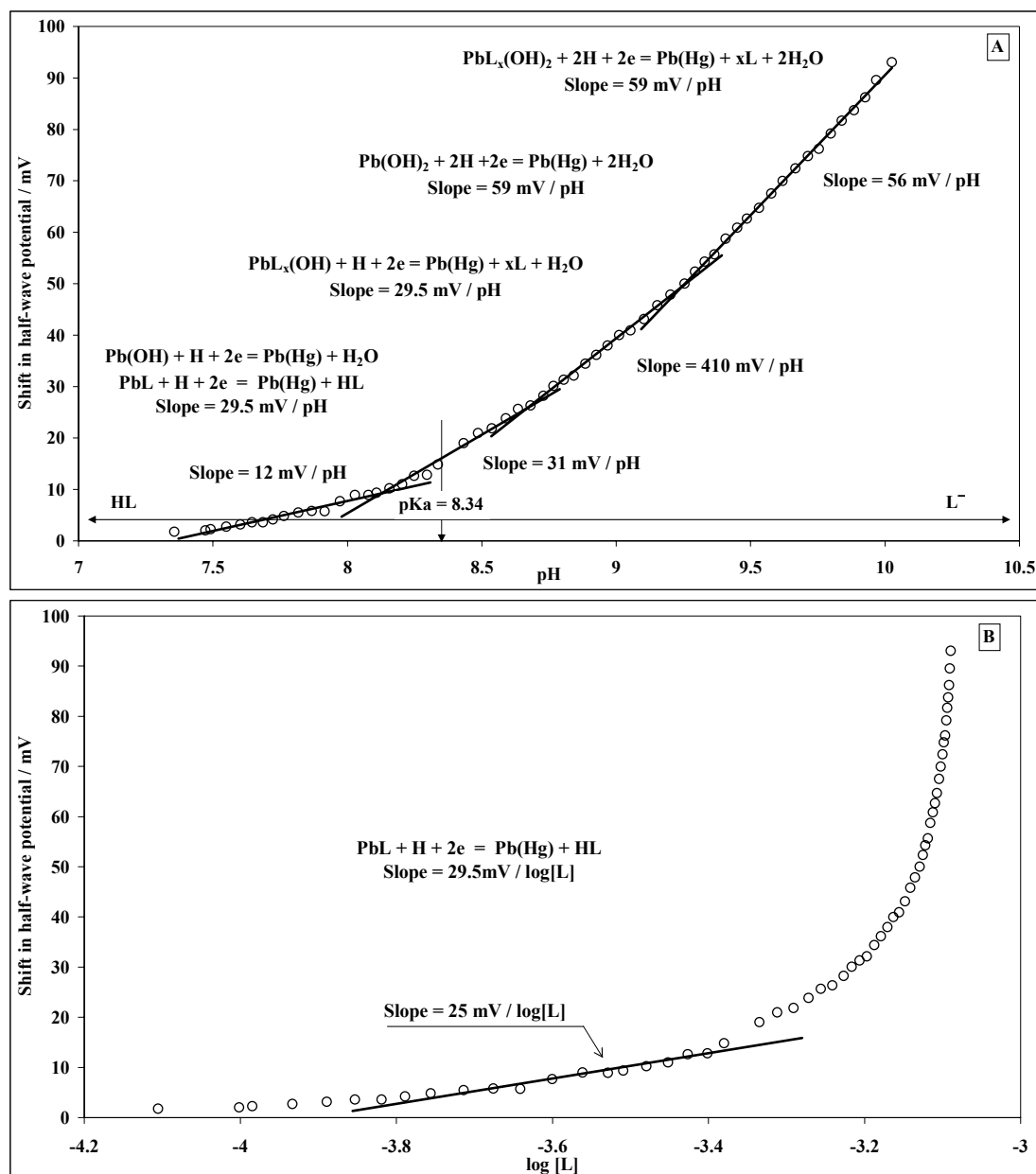


Figure 6-2 Interpretation of the DCP experimental data for $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system involving the corresponding slopes and electrochemical reactions at the DME electrode surface for $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 88.7$, $[\text{Pb}^{2+}] = 9.6 \times 10^{-6} \text{ M}$, 0.1 M KNO_3 at 25.0°C . Variation in the half-wave potential shift with the pH (A) and log [L] (B).

Next, a slope of 41 mV / pH was recorded between pH 8.6 and 9.3. In this pH range, the possible species present in solution are: $\text{PbL}_x(\text{OH})$ and $\text{PbL}_x(\text{OH})_2$ species ($x = 1$ and/or 2) and $\text{Pb}(\text{OH})_2$. The presence in solution of $\text{PbL}_x(\text{OH})$ and $\text{PbL}_x(\text{OH})_2$ species, where $x = 1$ or 2 , would result in theoretical slopes of 29.5 and 59 mV/pH, respectively. The reduction of the metal hydroxide, $\text{Pb}(\text{OH})_2$, should also be considered (see reactions in Figure 6-2A). However, the formation of this metal hydroxide species, as a major one, should be excluded since no precipitation was observed up to pH 10; this clearly indicates that the ligand must be involved in the complex formation with lead. The occurrence

of this non-nernstian slope, 41 mV / pH, is explained if the coexistence of all the hydroxide species, $\text{PbL}_x(\text{OH})_y$ (x and $y = 1$ and 2), in solution at this pH range is considered; the presence of $\text{PbL}_x(\text{OH})_2$ species in solution increases the theoretical slope to 59 mV/pH, and this slope was just observed above pH 9.3. The last region, between pH 9.3 and 10.0, is the region where $\text{PbL}_x(\text{OH})_2$ exists in higher amount in solution, but probably still some $\text{PbL}_x(\text{OH})$ species is present in solution since the experimental slope does not reach the theoretical one (Figure 6-2A).

The formation of PbL_n species was evaluated by the graphical analysis of the observed half-wave potential shift vs. $\log [L]$ (Figure 6-2B). The presence of PbL in solution was clearly evidenced since a slope of 25 mV / $\log [L]$, lower than the theoretical one (29.5 mV / $\log [L]$), was obtained. This species should be present in solution but not as major species. Figure 6-2B also indicates that no significant amount of PbL_2 is expected to be formed in solution. The formation of this metal complex, as a major species in solution, would generate a slope of 59 mV / $\log [L]$ in Figure 6-2B.

From the graphical analysis described above, the proposed model for $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system should include PbL and $\text{PbL}_x(\text{OH})_2$ species. In order to have a sequence of complexes, the species, PbL_2 and $\text{PbL}_x(\text{OH})$, should also be tested in the fitting procedures, but probably if they exist are minor species in solution.

6.3.3 MODELLING AND OPTIMIZATION OF THE STABILITY CONSTANTS: DCP & GEP

The modeling procedures and the optimization of the stability constants were performed using the ECF and CCF curves assuming different models shown in Table 6-1 and Figure 6-3. The curve for lead hydroxide species (dashed line) was also included in the figure. The comparison between the former curve (dashed line) and the ECFC clearly shows the occurrence of complexation between lead and TAPS; in the absence of TAPS complexation, precipitation would occur around pH 8.9 and that was not observed. It is also possible to see that the gap between the calculated curve for the lead hydroxide species and the experimental data was not very large; a maximum difference of about 18 mV was quantified. This means that, under these experimental conditions, hydroxide species assume an important role in the lead speciation of this system. Note that the departure between the calculated hydroxide curve and the experimental data only starts above pH 8.0, and up to pH 8.5 the difference between these two curves was only 4.6 mV, which means that the first slope identified in Figure 6-2A was mainly due to the formation of $\text{Pb}(\text{OH})$; PbL should be a minor species.

In order to have a sequence of formed species, the refinement procedures started with two simple models: Model 1: $\text{PbL} + \text{PbL}(\text{OH}) + \text{PbL}(\text{OH})_2$ and Model 2: $\text{PbL} + \text{PbL}_2 + \text{PbL}_2(\text{OH}) + \text{PbL}_2(\text{OH})_2$. The results obtained for both $[\text{L}_T]:[\text{Pb}_T]$ ratios are summarized in Table 6-1. For Model 2, PbL_2 was

always rejected; this fact is in agreement with the graphical analysis of Figure 6-2B and suggests that this species does not seem to exist in an appreciable extent under the experimental conditions used. For the lowest $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratio, 88.7, PbL was also rejected (Model 2, Table 6-1). This fact just evidences the previous statements that $\text{Pb}(\text{OH})$ is the major species up to 8.5 (Figure 6-3). The CCFC for Model 2 was included in Figure 6-3.

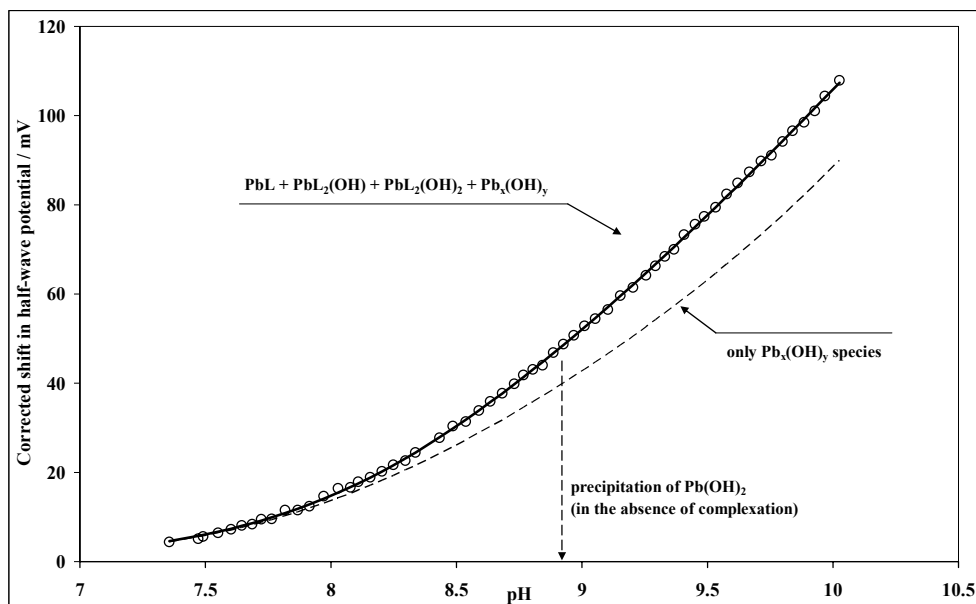


Figure 6-3 Experimental (circles) and calculated (lines) complex formation curves for $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system obtained for the models indicated in the figure for DCP data: $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 88.7$, $[\text{Pb}^{2+}] = 9.6 \times 10^{-6} \text{ M}$, at 25.0°C and 0.1 M KNO_3 . Values of the stability constants used for the models are summarized in Table 6-1. CCFC for $\text{Pb}_3(\text{OH})_3$ complexes was also included (dashed line).

The fit of Model 1 resulted in a higher standard deviation, and the fitting for the lower $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratio was only possible after fixing the value of PbL to the refined value obtained from Model 2, $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 124.3$ (Table 6-1). This fact corroborates the statement anticipated before that this species should be a minor one. From a statistical point of view, Model 2, $\text{PbL} + \text{PbL}_2(\text{OH}) + \text{PbL}_2(\text{OH})_2$, fitted the experimental results, for both $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratios, with lower standard deviation than Model 1, suggesting that Model 2 reproduces better the experimental data.

In order to provide more information, we decided to run GEP titrations. This technique, which works at much lower $[\text{L}_\text{T}]:[\text{M}_\text{T}]$ ratios can provide important information for the characterization of the system under study. Two $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratios were tested: 6.2 and 10.4, $[\text{Pb}_\text{T}] = 9.7 \times 10^{-4} \text{ M}$ for both ratios. For the first ratio, the titration was only possible to run up to pH 7.8 and for the second ratio, experimental points were collected up to pH 8.0 due to the precipitation of the metal hydroxide species. The adopted strategy for refinement of data is synthesized in Table 6-1.

Table 6-1 Refinement of the stability constants (as $\log \beta$) obtained for $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system by polarography ($[\text{L}_T]:[\text{Pb}_T] = 88.7$, $[\text{Pb}^{2+}] = 9.6 \times 10^{-6}$ M, 58 points collected between pH 7.4 and 10.0; $[\text{L}_T]:[\text{Pb}_T] = 124.3$, $[\text{Pb}^{2+}] = 2.4 \times 10^{-5}$ M, 63 points collected between pH 6.4 and 10.0) and by potentiometry ($[\text{L}_T]:[\text{M}_T] = 6.2$, $[\text{Pb}^{2+}] = 9.7 \times 10^{-4}$ M, two titrations, 59 points collected between pH 4.4 and 7.6; $[\text{L}_T]:[\text{Pb}_T] = 10.4$, $[\text{Pb}^{2+}] = 9.7 \times 10^{-4}$ M, two titrations, 253 points collected between pH 4.9 and 8.0).

Complexes	DCP experiments				GEP experiments			
	$[\text{L}_T]:[\text{Pb}_T] = 88.7$		$[\text{L}_T]:[\text{Pb}_T] = 124.3$		$[\text{L}_T]:[\text{Pb}_T] = 6.2$		$[\text{L}_T]:[\text{Pb}_T] = 10.4$	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
PbL $\text{Pb} + \text{L} \rightleftharpoons \text{PbL}$	2.94 (F)	R	2.94 ± 0.07	3.09 ± 0.04	3.14 ± 0.06	3.25 ± 0.04	3.20 ± 0.04	3.32 ± 0.02
PbL(OH) $\text{Pb} + \text{L} + \text{OH} \rightleftharpoons \text{PbL(OH)}$	8.5 ± 0.1	NI	8.89 ± 0.05	NI	9.51 ± 0.06	NI	9.54 ± 0.04	NI
PbL(OH)₂ $\text{Pb} + \text{L} + 2\text{OH} \rightleftharpoons \text{PbL(OH)}_2$	14.03 ± 0.01	NI	14.36 ± 0.01	NI	R	NI	R	NI
PbL₂ $\text{Pb} + 2\text{L} \rightleftharpoons \text{PbL}_2$	NI	R	NI	R	NI	R	NI	R
PbL₂(OH) $\text{Pb} + 2\text{L} + \text{OH} \rightleftharpoons \text{PbL}_2(\text{OH})$	NI	12.17 ± 0.04	NI	11.91 ± 0.03	NI	12.47 ± 0.05	NI	12.16 ± 0.03
PbL₂(OH)₂ $\text{Pb} + 2\text{L} + 2\text{OH} \rightleftharpoons \text{PbL}_2(\text{OH})_2$	NI	17.08 ± 0.02	NI	16.88 ± 0.01	NI	R	NI	R
SD / mV	0.436	0.185	0.594	0.411				
Hamilton R-factor					0.0723	0.0616	0.0595	0.0518

For DCP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (see Part II, Chapter 2, Section 2.3.3). For GEP, Hamilton R-factor stands for a statistical parameter generated by the program ESTA (see Part V, Chapter 10, Section 10.2.4.1). NI- not included; R – rejected; F – fixed value.

For both [L_T]:[Pb_T] ratios, the refinement of the Models 1 (PbL + PbL(OH) + PbL(OH)₂) and 2 (PbL + PbL₂ + PbL₂(OH) + PbL₂(OH)₂), resulted in the rejection of PbL_x(OH)₂ species, due to the lack of data points at high pH values. Even though, there were no experimental evidences of the formation of PbL₂ species, attempts to include it were made; for both ratios, the refinement operations always resulted in its rejection (Model 2, Table 6-1), indicating that, under the experimental conditions tested, no significant amount of this species should be formed in solution. Also, for both [L_T]:[Pb_T] ratios, the model constituted by PbL + PbL₂(OH) fitted better the experimental data than model constituted by PbL + PbL(OH) (Hamilton R-factor lower for Model 2; Table 6-1). However, the refined overall stability constant value for log β_{PbL₂(OH)} for [L_T]:[Pb_T] = 6.2, is higher than those refined for DCP ratios. This fact can be explained by the small amount of complex formed up to pH 7.8.

For GEP experimental data, the model constituted by PbL plus PbL₂(OH) was the model that explained better the experimental results.

It is important to emphasize the good agreement for PbL and PbL₂(OH) overall stability constants refined from GEP (PbL₂(OH) only from [L_T]:[Pb_T] = 10.4 and PbL from both ratios) and DCP data (Table 6-1). This fact evidenced that even though a slight adsorption of the ligand (less than 3% at pH 8.34) was predicted from ACP experiments (Figure 6-1), it did not influence the final results.

Gathering the information obtained from GEP and DCP data just described above, it is possible to say that the model for Pb-(TAPS)_x-(OH)_y system should include the species: PbL, PbL₂(OH) and PbL₂(OH)₂. In a first attempt, the following overall stability constants values for PbL, PbL₂(OH) and PbL₂(OH)₂ could be proposed: 3.2 ± 0.2, 12.2 ± 0.3, and 17.0 ± 0.1, respectively. Note that standard deviations indicate here the ranges in stability constants refined from Model 2 of all ratios tested by DCP and GEP (Table 6-1).

To test the proposed model, SDD were generated for [L_T]:[Pb_T] ratios 6.2 and 10.9 (GEP experimental conditions) and for the [L_T]:[Pb_T] 88.7 and 124.3 ratios (DCP experimental conditions) (Figure 6-4). The first analysis of the figure shows the existence of several polynuclear lead species for [L_T]:[Pb_T] ratios used in GEP conditions (Figure 6-4A and B). Figure 6-4C and D evidence that PbL is a minor species under DCP experimental conditions, namely for [L_T]:[Pb_T] = 88.7, as it was already anticipated before, whereas in GEP conditions, specifically for [L_T]:[Pb_T] ratio 10.4, it becomes a major species in the pH range between 6.5 and 7.4. This fact clearly explains why, for [L_T]:[Pb_T] = 88.7, it was not possible to refine the value for PbL and the refined values for [L_T]:[Pb_T] = 124.3 were more randomly and systematically lower than those refined by GEP. Due to that, for the final overall stability constant for PbL, only refined values coming from Model 2 of GEP were considered and a final value of 3.29 ± 0.04 was calculated. For [L_T]:[Pb_T] ratio 6.2, Figure 6-4A predicts, besides the formation of PbL₂(OH), the formation of a large amount of polynuclear species (Pb(OH), Pb₃(OH)₄, Pb₄(OH)₄ and Pb₆(OH)₈) prior the precipitation predicted to occur at about 7.8 and this was just

observed. This clearly shows that, for this ratio, no appropriate conditions were available to refine $\text{PbL}_2(\text{OH})$ accurately, when compared with SDD generated from DCP data (Figure 6-4D) and from $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ 10.4 ratio of GEP (Figure 6-4B), where this species is a major one. The final stability constant value for $\text{PbL}_2(\text{OH})$ was calculated considering the values coming from $[\text{L}_\text{T}]:[\text{Pb}_\text{T}]$ ratios 10.4, 88.7 and 124.3, which resulted in the final value (as $\log \beta$) 12.1 ± 0.1 . Figure 6-4 unequivocally shows that the stability constant for $\text{PbL}_2(\text{OH})_2$ was only possible to be refined from DCP conditions, with a final value of 17.0 ± 0.1 .

In conclusion, the final proposed model is PbL , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$ for which the following overall stability constants values, as $\log \beta$, are 3.29 ± 0.04 , 12.1 ± 0.1 , and 17.0 ± 0.1 , respectively.

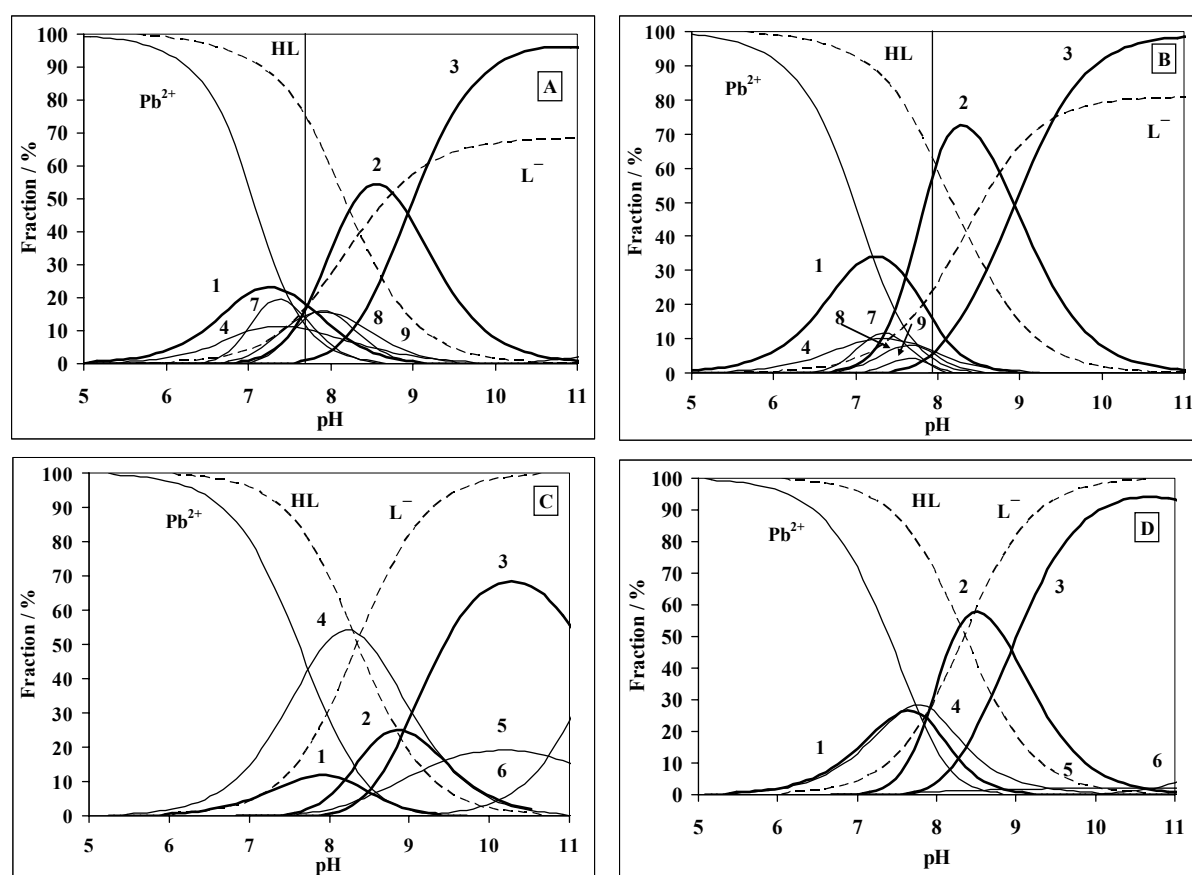


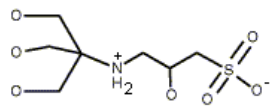
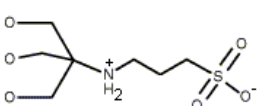
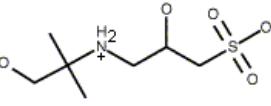
Figure 6-4 Species distribution diagrams computed for $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ system. GEP experimental conditions: $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 6.2$ (A) and 10.4 (B), with $[\text{Pb}^{2+}] = 9.7 \times 10^{-4}\text{M}$. DCP experimental conditions: $[\text{L}_\text{T}]:[\text{Pb}_\text{T}] = 88.7$ ($[\text{Pb}^{2+}] = 9.6 \times 10^{-6}\text{M}$) (C) and 124.3 ($[\text{Pb}^{2+}] = 2.4 \times 10^{-5}\text{M}$) (D). The metal-ligand model consisted of PbL (1), $\text{PbL}_2(\text{OH})$ (2), $\text{PbL}_2(\text{OH})_2$ (3), $\text{Pb}(\text{OH})$ (4), $\text{Pb}(\text{OH})_2$ (5), $\text{Pb}(\text{OH})_3$ (6), $\text{Pb}_4(\text{OH})_4$ (7), $\text{Pb}_3(\text{OH})_4$ (8), and $\text{Pb}_6(\text{OH})_8$ (9) for which stability constants, as $\log \beta$, were set to 3.2, 12.2, 17.0, 6.0, 10.3, 13.3, 35.5, 31.7, and 67.4, respectively.

6.3.4 COMPARATIVE EVALUATION OF Pb²⁺-(L)_x-(OH)_y SYSTEMS

TAPSO, TAPS and AMPSO are structural related and contain at least two hydroxyl groups in β carbons nearby to a secondary amino group (Table 6-2). Specifically, in the case of TAPSO and TAPS, the only difference between these two buffers is the fact that TAPSO has one more hydroxyl group than TAPS in the β carbon nearby the sulfonic group (Table 6-2).

No comparative analysis of lead complexation between TAPS and AMPSO can be done because the proper characterization of the model for Pb-(AMPSO)_x-(OH)_y system was not possible to be defined. Even using lower lead concentrations (about 10⁻⁵ M), as it can be used in polarography, the fact that the ligand deprotonates in the pH range where lead hydrolyses and precipitation of Pb(OH)₂ species occur, did not allowed a correct definition of the model.

Table 6-2 Stability constants (as log β) for Pb²⁺-(L)_x-(OH)_y systems.

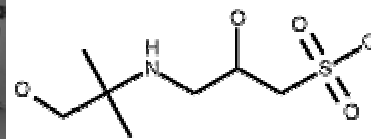
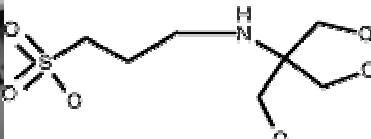
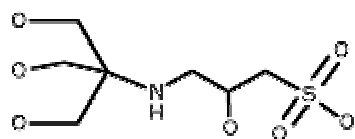
Ligand	TAPSO	TAPS	AMPSO
Structure			
pKa	7.55	8.342	9.05
Logβ _{Pb}	3.7	3.29	-
Logβ _{PbL(OH)}	-	-	-
Logβ _{PbL(OH)2}	-	-	-
Logβ _{PbL2}	6.6	-	-
Logβ _{PbL2(OH)}	12.9	12.1	-
Logβ _{PbL2(OH)2}	17.8	17.0	-
References	Part III, Chapter 5	This work	[19]

The comparison of the lead complexation properties observed with TAPSO (Part III, Chapter 5) and TAPS shows that lead binds stronger with TAPSO (Table 6-2). A simple evaluation based on the basicity of the two ligands points out to an enlargement in the stability constant values with the increase of the pKa [20], which was not observed (Table 6-2). The fact that PbL stability constants, for Pb-(TAPSO)_x-(OH)_y system, is 0.4 log unit higher than for Pb-(TAPS)_x-(OH)_y system, though the basicity of TAPSO is about 0.8 smaller than for TAPS, suggests that the TAPSO's hydroxyl group near the sulfonic group is involved in the coordination, forming a five-membered chelate ring and increasing the stability of the PbL complex. Comparing both models, it raises that PbL₂ species was not evidenced for Pb-(TAPS)_x-(OH)_y system. Because TAPS forms weaker complexes with lead than TAPSO and has a pKa₂ almost one unit more basic than TAPSO (8.34 and 7.55, respectively - Table 6-2), lead complexation (formation of PbL) starts to occur about one unit of pH latter than with TAPSO. Additionally, the fact that the deprotonation of the ligand occurs in the same pH range of lead hydrolysis favors the formation of PbL₂(OH) instead of PbL₂.

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PART IV MODELLING OF $\text{Cd}-(\text{BUFFER})_x-(\text{OH})_y$ AND $\text{Zn}-(\text{BUFFER})_x-(\text{OH})_y$ SYSTEMS

- 7 INTERPRETATION OF NON-NERNSTIAN SLOPES IN GRAPHIC ANALYSIS OF DATA COLLECTED IN pH-RANGE CLOSE TO DEPROTONATION OF A LIGAND. PART I. A GLASS ELECTRODE POTENTIOMETRIC AND POLAROGRAPHIC STUDY OF $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ AND $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ SYSTEMS⁶

ABSTRACT

In this work, the complexation of cadmium and zinc ions by TAPSO, a commercial biological buffer, was evaluated using three electrochemical techniques, at fixed total-ligand and total-metal concentration ratio and varied pH, at 25.0 ± 0.1 °C and ionic strength set to 0.1 M KNO_3 . For both metal-ligand systems, complexation was evidenced in the pH-range close to deprotonation of the ligand and the final models were optimised after a meticulous graphical analysis. For $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ system, two complexes, CdL and CdL_2 , were identified in the buffering region of the ligand. The proposed final model for this system is: CdL , CdL_2 and $\text{CdL}_2(\text{OH})$ with stability constants, as $\log \beta$, of 2.2, 4.2 and 8.6, respectively. For $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ system, the complex ZnL is the main species formed in the buffering pH range. The proposed final model is ZnL , $\text{ZnL}(\text{OH})$ and $\text{ZnL}(\text{OH})_2$ with overall refined stability constants (as $\log \beta$) to be: 2.5, 7.2 and 13.2, respectively.

7.1 INTRODUCTION

The choice of alternative buffers had greatly increased with the commercial availability of the zwitterionic N-substituted aminosulfonic acids synthesized by Good and co-workers [1-3]. Good's buffers have, in fact, been widely adopted and at least one major chemical supplier devotes a special section of its catalogue to them (*e.g.*, from Sigma – Aldrich).

In generating a series of more than twenty buffers for use in biological studies, these workers listed the prevention of metal ion complexation as one of their desired goals. However, most of a broad series of these buffers have been shown to cause metal ion interference as a result of complexation. Therefore, the knowledge of the stability constants of (Good buffer)-(metal ion) complexes is essential for accurate metal ions speciation studies in natural waters, biological fluids or industrial processes, when these biological buffers are required to be used.

As far as we know, no reports regarding studies of the interaction between TAPSO and cadmium

⁶ Talanta 68 (2006) 819-830

are described in the literature. For zinc, there are some reports regarding studies of the ternary complexes of biological importance [4-6], but an exhaustive speciation study of $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ system has never been performed.

In previous studies, we have studied the interaction between TAPSO and copper (Part II, Chapter 2) and lead (Part III, Chapter 5). These works evidenced that TAPSO forms several strong complexes with these metal ions in the pH buffer range, and thus, can decrease strongly the free metal ion concentration, when this ligand is used as a buffer. In this context, we think that the study of the interaction of TAPSO with cadmium and zinc may furnish a further contribution to obtain a systematic information of the overall stability constants of the (TAPSO)-(metal ion) systems where complexation occurs.

In the present study, the behaviour of solutions containing TAPSO and cadmium or zinc was studied by three electrochemical techniques: DCP, DPP and GEP, at fixed total ligand to total metal concentration ($[\text{L}_\text{T}]:[\text{M}_\text{T}]$) ratios of various orders of magnitude and various pH values, at 25 °C and 0.1 M KNO_3 ionic strength. The final metal–ligand models for the two systems were obtained after a meticulous graphical analysis. The quasi-reversibility of the electrochemical reduction of zinc ion was fruitfully corrected with a simple procedure.

7.2 EXPERIMENTAL

7.2.1 MATERIALS AND REAGENTS

For polarography, a stock solution of cadmium, 4.0×10^{-2} M, was prepared from the analytical grade salt ($\text{Cd}(\text{NO}_3)_2$, Merck) at pH 2.3 (addition of standardized nitric acid) and the ionic strength was adjusted to 0.1 M. For potentiometry, a Merck standard solution of cadmium (9.0×10^{-3} M) was used. An acidic standard solution of zinc (1.5×10^{-2} M), purchased from Merck, was used for both techniques.

Additional information about the remaining reagents and experimental conditions were described previously (Part II, Chapter 2, Section 2.2.1).

7.2.2 APPARATUS

All measurements reported in this work were performed on solutions adjusted to ionic strength 0.1 M KNO_3 in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer,

and thermostatted at 25.0 ± 0.1 °C using a water bath.

7.2.2.1 POLAROGRAPHY

The polarographic measurements of Cd-(TAPSO)_x-(OH)_y system were performed as described previously (Part II, Chapter 3, Section 3.2.2.2).

Polarographic measurements of Zn-(TAPSO)_x-(OH)_y system were performed as described previously (Part II, Chapter 4, Section 4.2.2.1).

7.2.2.2 POTENTIOMETRY

Potentiometric titrations of Cd-(TAPSO)_x-(OH)_y and Zn-(TAPSO)_x-(OH)_y systems were performed as previously described (Part II, Chapter 2, Section 2.2.2.2).

7.2.3 PROCEDURE

7.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

For all techniques, the calibration of the glass electrodes (measurements of pH as $-\log [H^+]$), was achieved as previously described (Part II, Chapter 3, Section 3.2.3.1).

7.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

In order to evaluate the adsorption of the ligand on the surface of the mercury working electrode, ACP polarograms were run in the absence of the metal ion. The experimental conditions used were described elsewhere (Part II, Chapter 2, Section 2.2.3.2).

7.2.3.3 DIRECT CURRENT AND DIFFERENTIAL PULSE POLAROGRAPHY

For both systems, Cd-(TAPSO)_x-(OH)_y and Zn-(TAPSO)_x-(OH)_y, two polarographic techniques (DC and DP) were performed on the same sample solution. The procedure used to run the polarographic titrations was described elsewhere (Part II, Chapter 2, Section 2.2.3.3); a set of 40 and

30 polarograms (representing all the species present at each pH value) were recorded for Cd-(TAPSO)_x-(OH)_y and Zn-(TAPSO)_x-(OH)_y systems, respectively. Several [L_T]:[M_T] ratios were used for each system. For Cd-(TAPSO)_x-(OH)_y system, two different [L_T]:[Cd_T] ratios were evaluated, 186 and 308, with total cadmium concentration of 4.0×10^{-5} and 5.0×10^{-5} M, respectively. In the DPP measurements, a pulse height of 50 mV was used, with a pulse width and an integration time of 200 ms and 60 ms, respectively. A drop time of 2 s and a step potential of 4 mV were used for both techniques (DC and DPP). For Zn-(TAPSO)_x-(OH)_y system, two [L_T]:[Zn_T] ratios were evaluated, 150 and 255, with total zinc concentrations of 5.2×10^{-5} and 2.8×10^{-5} M, respectively. A drop time of 1 s and a step potential of 4 mV were used. For DPP mode, a modulation time of 20 ms and modulation amplitude of 50 mV were used. The equilibrium of the metal - ligand solutions was tested and it was reached in a few minutes.

7.2.3.4 GLASS ELECTRODE POTENTIOMETRY

For Cd-(TAPSO)_x-(OH)_y system, seven titrations, using three independent solutions, were performed in the pH range between 4.0 and 9.0. For each titration, about 50 points were collected and a [L_T]:[Cd_T] ratio of 3.3 with a total cadmium concentration of 3.0×10^{-3} M was used. For Zn-(TAPSO)_x-(OH)_y system, five titrations, using two independent solutions, were carried out. For each titration, about 45 points were collected in the pH range between 3.0 and 7.5 and a [L_T]:[Zn_T] ratio of 3.3 with a total zinc concentration of 7.6×10^{-4} M was used. For both systems, monotonic additions of standardised KOH were done.

7.2.4 DATA TREATMENT

The simulation and optimization procedures of potentiometric data was done using ESTA program [7, 8]; for more details about the program see Part II, Chapter 2, Section 2.3.3 and Part V, Chapter 10, Section 10.2.4.1.

The refinement of polarographic data was performed using the Cukrowski method [9, 10]; as it was described previously (Part II, Chapter 2, Section 2.2.4).

During the refinement of the overall stability constants for both systems, the water [11] and ligand (Part II, Chapter 2, Table 2-1) protonation constants, as well as all known stability constants for M_x(OH)_y species [11] (Table 7-1), were kept fixed.

Table 7-1 Overall stability constants for Cd²⁺ and Zn²⁺ complexes with OH⁻, at 25 °C [11].

	Equilibrium	Log β	I (M)
Cadmium	$\text{Cd}^{2+} + \text{OH}^- \rightleftharpoons \text{Cd}(\text{OH})^+$	4.3	3.0
	$\text{Cd}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Cd}(\text{OH})_2$	7.7	3.0
	$\text{Cd}^{2+} + 3\text{OH}^- \rightleftharpoons \text{Cd}(\text{OH})_3^-$	10.3	3.0
	$\text{Cd}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Cd}(\text{OH})_4^{2-}$	12.0	3.0
	$4\text{Cd}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Cd}_4(\text{OH})_4^{4+}$	24.9	3.0
	$\text{Cd}(\text{OH})_2 (\text{s}) \rightleftharpoons \text{Cd}^{2+} + 2\text{OH}^-$	-14.29	3.0
Zinc	$\text{Zn}^{2+} + \text{OH}^- \rightleftharpoons \text{Zn}(\text{OH})^+$	4.7	0.1
	$\text{Zn}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Zn}(\text{OH})_2$	(10.2)	0.0
	$\text{Zn}^{2+} + 3\text{OH}^- \rightleftharpoons \text{Zn}(\text{OH})_3^-$	13.9	0.0
	$\text{Zn}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Zn}(\text{OH})_4^{2-}$	15.5	0.0
	$4\text{Zn}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Zn}_4(\text{OH})_4^{4+}$	27.9	3.0
	$\text{Zn}(\text{OH})_2 (\text{s}) \rightleftharpoons \text{Zn}^{2+} + 2\text{OH}^-$	-14.82	0.1

7.3 RESULTS AND DISCUSSION

7.3.1 LIGAND ADSORPTION AND METALS REVERSIBILITY STUDIES

Ligand adsorption studies, reported in a previous chapter (Part II, Chapter 2, Section 2.3.2), have shown that in the potential region where cadmium and zinc are reduced, no significant decrease of the capacitive current was observed in the concentration range studied ($[\text{TAPSO}] < 1.3 \times 10^{-2} \text{ M}$).

The polarograms recorded for Cd-(TAPSO)_x-(OH)_y system indicated that the electrochemical process was fully reversible. For both polarographic titrations, fully reversible DC polarograms were recorded (gamma coefficient, which indicates the steepness of polarographic waves and whose theoretically value should be one for a reversible process, varied between 0.96 and 1.0) and DPP peak width, $w_{1/2}$, was constant throughout the experiment, $60.9 \pm 0.1 \text{ mV}$ (as predicted for a reversible process [12]).

For Zn-(TAPSO)_x-(OH)_y system, the metal reduction process at the mercury electrode surface was not fully reversible. A decrease in the DC reversibility (decrease in the steepness of the polarographic wave from 0.94 to 0.65), in the pH range between 5.0 and 9.0, was observed. In Figure 7-1, DCP waves of the metal zinc alone and of the metal in the presence of the ligand at pH values between pH 4.9 and 9.5 are plotted. As it is evidenced in figure, the irreversibility of the system increased strongly (wave steepness lowered down to below 0.4) for pH values above 9. In order to use the data for this system and since the limiting diffusion current I_d does not depend on the degree of reversibility of the electrochemical process, a correction for the departure from the reversibility of the recorded polarographic curves up to pH 9 was done. This procedure, described in a previous work (Part II, Chapter 3, Section 3.3.1), corrects the effect of the quasi-reversibility by fixing the gamma

coefficient at value of 1 and computes the corrected limiting diffusion current and potential of half height, $E_{1/2}$, for electrochemically reversible process.

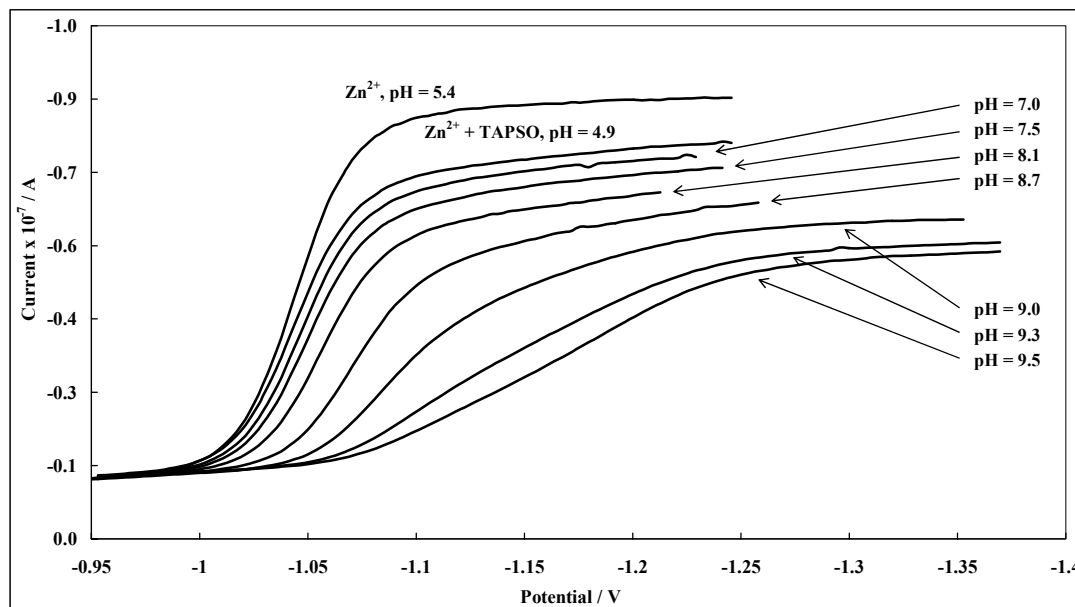


Figure 7-1 Typical DC polarograms of zinc in the presence of TAPSO with the increase of pH.. No correction for dilution was made (curves are seen as recorded).

For DPP and for ratio $[L_T]:[Zn_T] = 255$, the $w_{1/2}$ varied between 63.0 (metal alone) and 74.9 mV, with the increase of pH; a similar behaviour was obtained for $[L_T]:[Zn_T] = 150$. Attempts to correct DPP experimental curves, as described in Part II, Chapter 2, Section 2.3.3, Equations 2-5 to 2-7, was not successful. Therefore, for $Zn-(TAPSO)_x-(OH)_y$ system, only DCP data were evaluated.

7.3.2 MODELLING OF $Cd-(TAPSO)_x-(OH)_y$ SYSTEM AND REFINEMENT OF STABILITY CONSTANTS

The analysis of polarographic titrations (DC and DP polarography) data in a pH range between 5.0 and 9.5 using different $[L_T]:[Cd_T]$ ratios showed that $Cd-(TAPSO)_x-(OH)_y$ system behaved as labile and dynamic on the polarographic time scale; only one peak corresponding to all labile species present in solution, which shifted to more cathodic potential values, was observed (data not shown). The analysis and discussion presented below is related to the DPP data.

The prediction of the metal-ligand species present in a solution was achieved by graphical analysis [10]; the plot of the peak potential as a function of the pH of the solution is presented in Figure 7-2A. Protonation constant for the ligand and the different forms of the ligand (HL and L^-) according to the pH, are also marked in Figure 7-2A. Four different regions were identified with the variation of pH. In

the first region, up to pH = 6.5, complexation was negligible; only 1.8 mV shift was observed. In region II, a slope of about 10 mV per pH unit was observed, which is smaller than one would expect for any metal containing species of a CdL type. Theoretical slope for CdL (one proton in the electrochemical reaction) would be (charges were omitted for sake of simplicity):



But, between pH 6.5 and 8.5, the reaction with no proton involvement should also be considered:

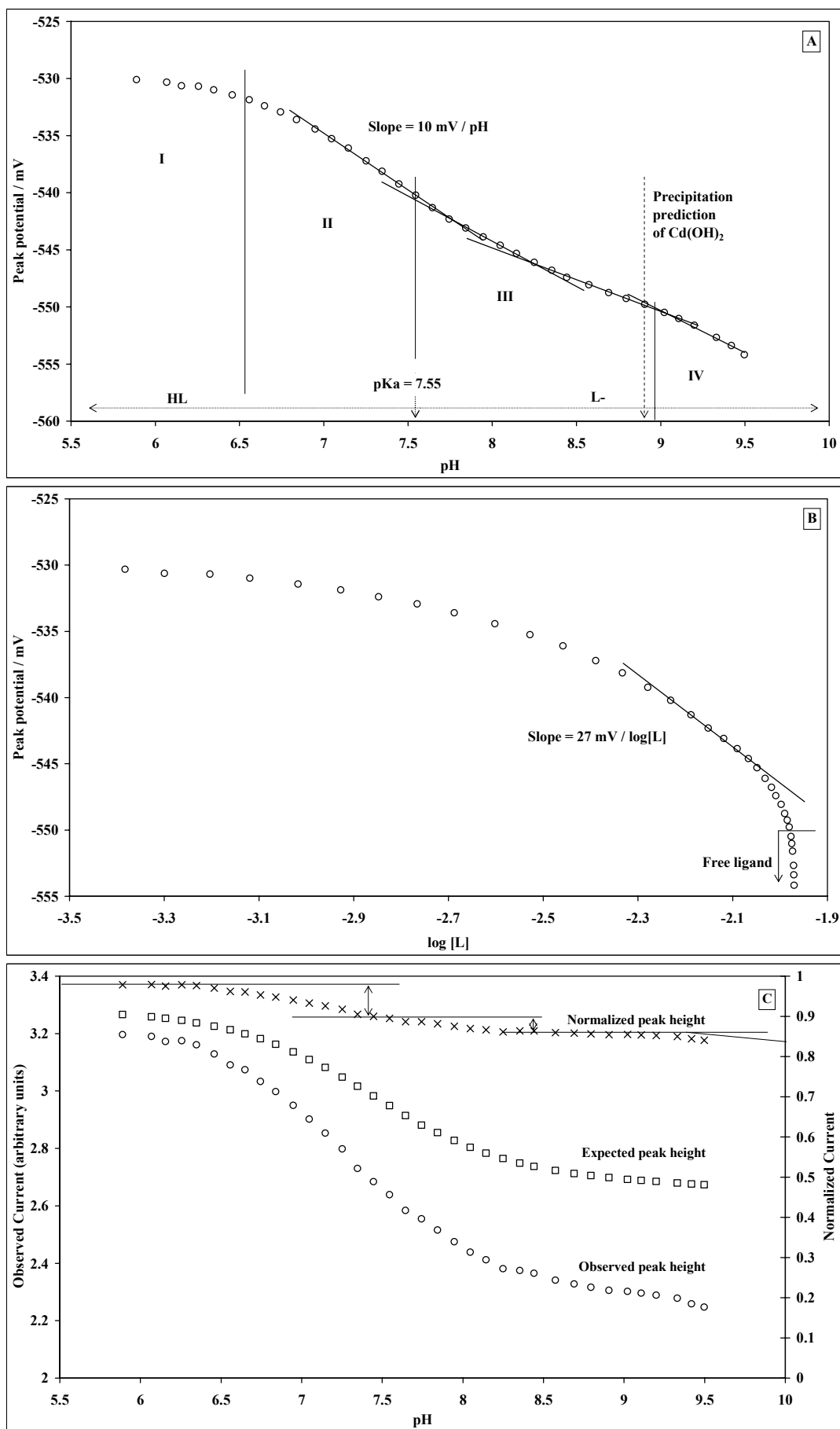


and this is probably the explanation why the slope has never developed to its theoretical value. So, CdL must be formed even though the slope did not suggest that. In region III, above pH 7.55, there was a decrease in the slope, followed by another drop in the slope around pH 8.2. For pH values higher than the pK_a , the ligand is mainly in the deprotonated form, which means that the main reduction reaction of CdL, at the DME, is the second reaction described above (no proton involved). This caused the decrease in the slope. The second drop must be due to the formation of other species. If only CdL would be formed and present as a major metal containing species in the pH range between 8 and 9 then there should be no slope observed due to the fact that the ligand is almost entirely in its free form. The fact that the slope does not reach a value of zero (still small slope is observed between pH of 8.5 and 9) must indicate the formation of another species. The other species most likely would be CdL₂. It is important to emphasize that one must not expect the theoretical slope of about 59 mV/pH since we consider the data in the pH range where ligand is almost fully deprotonated. Moreover, the formation of OH containing species below pH 9 would make the slope much more pronounced. For instance, the formation of Cd(OH) and/or CdL_x(OH), would increase the slope significantly, since the reduction at the DME electrode generates a slope of about 30 mV according to the following reaction:



The final increase in the slope, above pH of 9.0, clearly indicated the formation of CdL_x(OH) species (region IV).

The analysis of the observed peak potential versus log [L] (Figure 7-2B) confirmed the formation of CdL (the slope reached a value of about 30 mV per log unit) in the pH range between 7.4 and 8.4. Assuming the formation of CdL₂, one would not observe the theoretically expected slope of about 59 mV/log [L] because this complex is formed in the pH range where there is no further increase in the free ligand concentration (see last points that aim at a fixed value of the free ligand concentration).



In Figure 7-2C, the variation of the peak height with pH is represented. The smooth drop in the peak height indicates the formation of complexed species between the metal and the ligand. A careful analysis of the normalized peak height corroborated the formation of the species mentioned above. The normalized current started to decrease around pH 6.5, where complexation started, as it is evidenced in Figure 7-2A. At pH 7.5, the normalized peak height had a total drop of about 0.1 units due to the formation of CdL . Then, the normalized peak height continues to decrease gradually. At pH ≈ 8.2 , the observed drop in the normalized peak height is explained by the beginning of formation of CdL_2 . Around pH 9, the hydroxide species starts to be formed and this explains further small drop in the normalized peak height.

From the graphical analysis described above, the $\text{Cd-(TAPSO)}_x\text{-(OH)}_y$ model should include CdL , CdL_2 and $\text{CdL}_x(\text{OH})$ species.

In Figure 7-3, the ECFC for $[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 308$ and $[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 186$, as well as the CCFC for $\text{Cd}_x(\text{OH})_y$ species, are included. The comparison of the CCFC for $\text{Cd}_x(\text{OH})_y$ (— - —) with both ECFC clearly evidences complexation between TAPSO and cadmium. At pH 8.0, if no complexation with TAPSO occurred, the shift of the potential should be zero, as it is evidenced by the CCFC for $\text{Cd}_x(\text{OH})_y$ species, but a shift of 10 mV was observed, for the lowest ratio. In addition, precipitation of $\text{Cd}(\text{OH})_2$, in the absence of TAPSO complexation, was predicted around pH 8.9 and this was not confirmed experimentally. In conclusion, these facts evidence that it was not possible to make such a large experimental error and clearly confirms that TAPSO forms complexes with cadmium. In addition, it is also observed that the CCFC for $\text{Cd}_x(\text{OH})_y$ species is parallel to both ECFC at highest pH values. This indicates that the metal hydroxide species, $\text{Cd}_x(\text{OH})_y$, and the $\text{CdL}_x(\text{OH})_y$ species are formed in the same pH region.

← **Figure 7-2** Graphic analysis of the data obtained for the $\text{Cd-(TAPSO)}_x\text{-(OH)}_y$ system by DPP. (A) – E_p vs. pH; (B) – E_p vs. $\log [\text{L}]$; (C) – I_p vs. pH. $[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 308$, $[\text{Cd}^{2+}] = 5.0 \times 10^{-5}$ M.

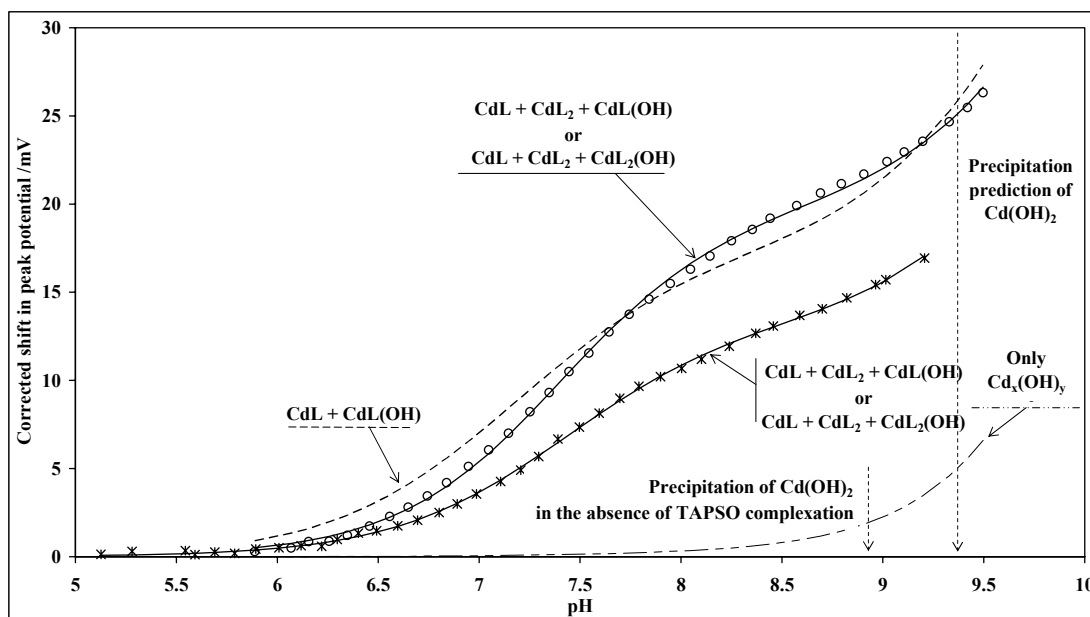


Figure 7-3 Modelling of the $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ system by use of complex formation curves. Circles and asterisks represent the experimental complex formation curves for $[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 308$ (open circles), $[\text{Cd}^{2+}] = 5.0 \times 10^{-5} \text{ M}$ and $[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 186$ (asterisks), $[\text{Cd}^{2+}] = 4.0 \times 10^{-5} \text{ M}$, respectively. The fitted models are indicated in the figure (CCFC are seen as solid or dashed lines) and the refined stability constants are summarized in Table 7-2.

Next, a special procedure that was based on simulation of CCFC assuming several models and comparing them with the ECFC curves was used to propose the final model (Figure 7-3). The strategy used is summarized in Table 7-2.

Even though the graphic analysis in Figure 7-2 has suggested that CdL_2 would probably be present in the solution (Figure 7-2A and C), we decided to test a simpler model by fitting CdL with $\text{CdL}(\text{OH})$ into the experimental data (Model 1 in Table 7-2 and Figure 7-3). However, when the stability constants for these two species were refined, it became obvious that more species must be formed. Statistically, the refined $\text{CdL} + \text{CdL}(\text{OH})$ model had an excellent standard deviation for both ratios, namely for the lower one. However, the graphical analysis of the fitting revealed that this model was not the correct one, as it is exemplified for the highest ratio in Figure 7-2. The CCFC for this model (dashed line in Figure 7-3) had two cross-points, at pH 7.7 and 9.2. Between pH 6.0 and 7.7, the CCFC was above the experimental data (ECFC); this means that the computed stability constant for CdL , the main species present in solution in this pH range, was too large; $\log K_1$ must be smaller than 2.4. Between pH 7.7 and 9.2, the CCFC was below the ECFC; this fact indicated that even though $\log K_1$ was too large, the complex CdL could not make the observed shift difference, suggesting that another species, not containing the OH group, was missing in the metal-ligand model.

Table 7-2 Overall stability constants (as $\log \beta$ values) for $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ system determined at 25 °C and $\mu = 0.1$ M by DPP for $[\text{L}_T]:[\text{Cd}_T] = 186$, $[\text{Cd}^{2+}] = 4.0 \times 10^{-5}$ M (38 points, between pH 5.1 and 9.2) and for $[\text{L}_T]:[\text{Cd}_T] = 308$, $[\text{Cd}^{2+}] = 5.0 \times 10^{-5}$ M (36 points, between pH 5.9 and 9.5) and by GEP for $[\text{L}_T]:[\text{Cd}_T] = 3.3$, $[\text{Cd}^{2+}] = 3.0 \times 10^{-3}$ M (88 points, two titrations, from pH 4.0 to 8.0).

Complexes	Equilibrium	DPP [L_T] : [Cd_T] = 186			DPP [L_T] : [Cd_T] = 308			GEP [L_T]:[Cd_T]=3.3
		Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	
CdL^+	$\text{Cd}^{2+} + \text{L}^- \rightleftharpoons \text{CdL}^+$	2.41 ± 0.02	2.30 ± 0.07	2.30 ± 0.07	2.42 ± 0.01	2.14 ± 0.07	2.14 ± 0.07	2.31 ± 0.02
CdL_2	$\text{Cd}^{2+} + 2\text{L}^- \rightleftharpoons \text{CdL}_2$	NI	4.2 ± 0.2	4.2 ± 0.2	NI	4.27 ± 0.07	4.28 ± 0.07	4.40 ± 0.03
$\text{CdL}(\text{OH})$	$\text{Cd}^{2+} + \text{L}^- + \text{OH}^- \rightleftharpoons \text{CdL}(\text{OH})$	6.7 ± 0.1	6.4 ± 0.3	NI	6.83 ± 0.04	6.59 ± 0.09	NI	NI
$\text{CdL}_2(\text{OH})^-$	$\text{Cd}^{2+} + 2\text{L}^- + \text{OH}^- \rightleftharpoons \text{CdL}_2(\text{OH})^-$	NI	NI	8.6 ± 0.3	NI	NI	8.55 ± 0.09	R
SD / mV		0.107	0.013	0.013	1.1701	0.0418	0.0415	
Hamilton R-factor								0.027

For DPP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (Part II, Chapter 2, Section 2.3.3). For GEP, Hamilton R-factor stands for a statistical Hamilton R-factor generated by the program ESTA (Part V, Chapter 10, Section 10.2.4.1). NI - not included; R – rejected.

Above pH 9.2, the CCFC was above the ECFC, which indicates that the calculated $\log \beta_{\text{CdL(OH)}}$ was too large.

In the next step, we included CdL_2 in the model (Table 7-2, Model 2), as it was suggested from the graphical analysis of Figure 7-2 and discussion above related to CCFC for the model containing CdL and CdL(OH) . This resulted in a very good fitting with a low overall standard deviation for both $[\text{L}_\text{T}]:[\text{Cd}_\text{T}]$ ratios. If one assumes that CdL(OH) should be a product of hydrolysis of the complex CdL, then the value of the refined stability constant for CdL(OH) was almost as expected from hydrolysis ($\log \beta_{\text{CdL(OH)}} = \log \beta_{\text{CdL}} + \log \beta_{\text{Cd(OH)}} = 2.3 + 4.3 = 6.6$).

If one accepts the strategy the simpler model the better, when refinement of data is concerned, then the model CdL, CdL_2 and CdL(OH) could be accepted. On the other hand, one might assume that the hydrolysis of CdL_2 may also take place. Hence, it was decided to incorporate this complex in the model; the refinement operations of the data, for which CdL, CdL_2 and $\text{CdL}_2(\text{OH})$ were included in the model (Table 7-2, Model 3) gave a similar fitting as that obtained for Model 2 (Figure 7-3, solid line). These facts showed that, as expected, it was not possible to distinguish between CdL(OH) and $\text{CdL}_2(\text{OH})$; note that these complexes are formed exactly in the same pH range where the ligand is fully deprotonated, and the same slope is obtained both complexes require the same amount of protons:



From the above it follows that two complexes, namely CdL, and CdL_2 , were identified with high certainty and their stability constants did not vary much when different hydroxo complexes $\text{CdL}_x(\text{OH})_y$ were incorporated in the metal – ligand model. In addition, the complexes CdL and CdL_2 are most important when buffering pH-range of the ligand TAPSO is of interest. It is impossible, for reason given above, to distinguish between CdL(OH) and $\text{CdL}_2(\text{OH})$, regardless of analytical technique used as these complexes are formed where there is not a protonated form of the ligand present; an increase in pH does not change the free ligand concentration. These two complexes, CdL(OH) and $\text{CdL}_2(\text{OH})$, describe the system equally well and since they are formed outside the buffering pH-range, they are not of importance in this type of the study.

Modelling of the solution composition for potentiometric analytical conditions (using the models and stability constants obtained from polarography) suggested that it should be possible to establish CdL and CdL_2 stability constants prior to precipitation. It was also predicted that no information about $\text{CdL}_x(\text{OH})_y$ species would be available from the GEP experiments (data not shown). Based on the polarographic results and predictions made for the best analytical conditions, GEP experiments were performed. Precipitation was observed at pH 8.0. The refined results for data collected between pH 4.0

and 8.0 from two titrations are summarized in Table 7-2. Similar results were obtained with the other titrations. When the model CdL, CdL₂ and CdL₂(OH) was investigated then the complex CdL₂(OH) was always rejected in the refinement operations or resulted in unreasonable stability constant values. The stability constant values obtained for CdL and CdL₂ were very comparable with the ones obtained by DPP, being the differences within the experimental errors.

Taking into account the different ratios and the different techniques performed, the following values for the stability constants (as log β values) are proposed for the complexes formed in the buffering pH region: CdL = 2.2 ± 0.2 , CdL₂ = 4.2 ± 0.2 , and for most likely hydroxo species, CdL(OH) = 6.4 ± 0.2 and CdL₂(OH) = 8.6 ± 0.2 (note that the standard deviations indicate here the ranges in stability constants obtained from different ratios and techniques and are within the experimental errors associated with both techniques).

Species distribution diagrams for model CdL, CdL₂ and CdL₂(OH), for [L_T]:[Cd_T] = 308 and for [L_T]:[Cd_T] = 3.3 were plotted in Figure 7-4. This figure shows that CdL and CdL₂ are the major species in solution in the buffering pH range (between pH 6.5 and 8.5) (a similar behaviour was obtained for [L_T]:[Cd_T] = 186, data not shown); only 37 % of CdL₂(OH) was predicted at pH 9.5, the highest pH for which experimental points were collected for [L_T]:[Cd_T] = 308 in DCP experiments. Figure 7-4 also evidences that, for GEP experimental conditions, no significant amount of CdL₂(OH) was formed up to pH 8.0, which explains why this species was rejected in the GEP refinement operations. For model CdL, CdL₂ and CdL(OH), similar species distribution diagrams were drawn, resulting in the same conclusions (data not shown).

The comparison of the values of the stability constants determined for Cd-(TAPSO)_x-(OH)_y in this work with other β -alcohols-amino ligands reported in the literature (triethanolamine, 2-amino-1-propanol, 2-amino-1-butanol, 2-amino-1-pentanol, 2-amino-1,3-propanediol) [11, 13] evidenced that the stability constants for CdL and CdL₂ are of the same order of magnitude.

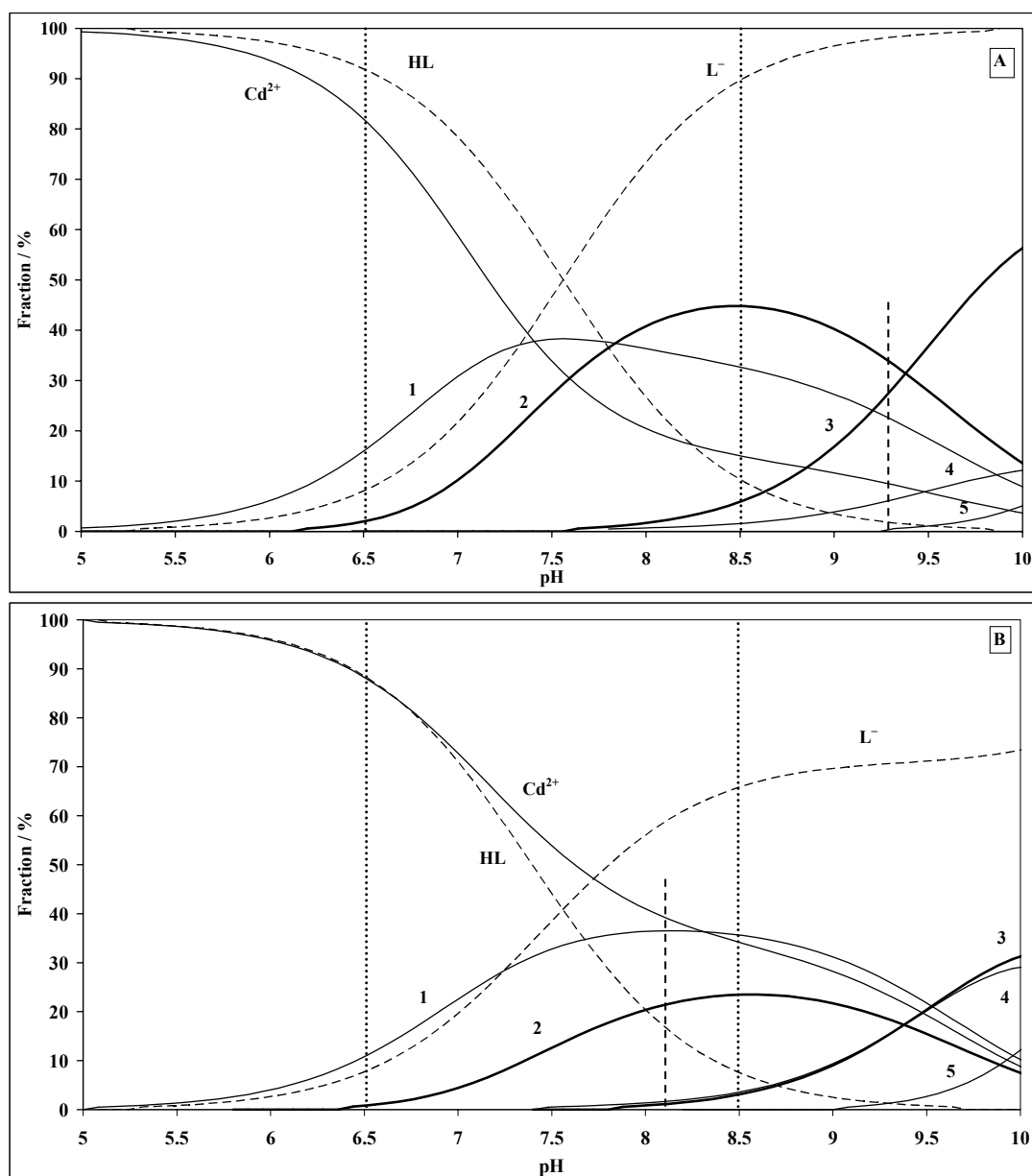


Figure 7-4 Species distribution diagrams for Cd-(TAPSO)_x-(OH)_y system, computed for (A) [L_T]:[Cd_T] = 308, [Cd²⁺] = 5.0 × 10⁻⁵ M and (B) [L_T]:[Cd_T] = 3.3, [Cd²⁺] = 3.0 × 10⁻³ M. The Cd-(TAPSO)_x-(OH)_y model used was: CdL (1), CdL₂ (2) and CdL₂(OH) (3), for which stability constant values, as log β, were 2.2, 4.2 and 8.6, respectively. Cd_x(OH)_y species were also considered (Table 7-1): Cd(OH) (4), Cd(OH)₂ (5). Dashed line represents Cd(OH)₂ precipitation and the pH range bounded by the dotted lines indicates the pH buffering region.

7.3.3 MODELLING OF $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ SYSTEM AND REFINEMENT OF STABILITY CONSTANTS

Figure 7-1 evidences that $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ system behaves as fully labile, since only one DC wave was registered in the pH range studied, corresponding to all the species present in solution.

After the correction of the waves regarding reversibility (for more details see Part II, Chapter 3, Section 3.3.1), the corrected half-wave potential as a function of pH was plotted (Figure 7-5). The different forms of the ligand according to the pH range and TAPSO's pK_a value were also marked in Figure 7-5.

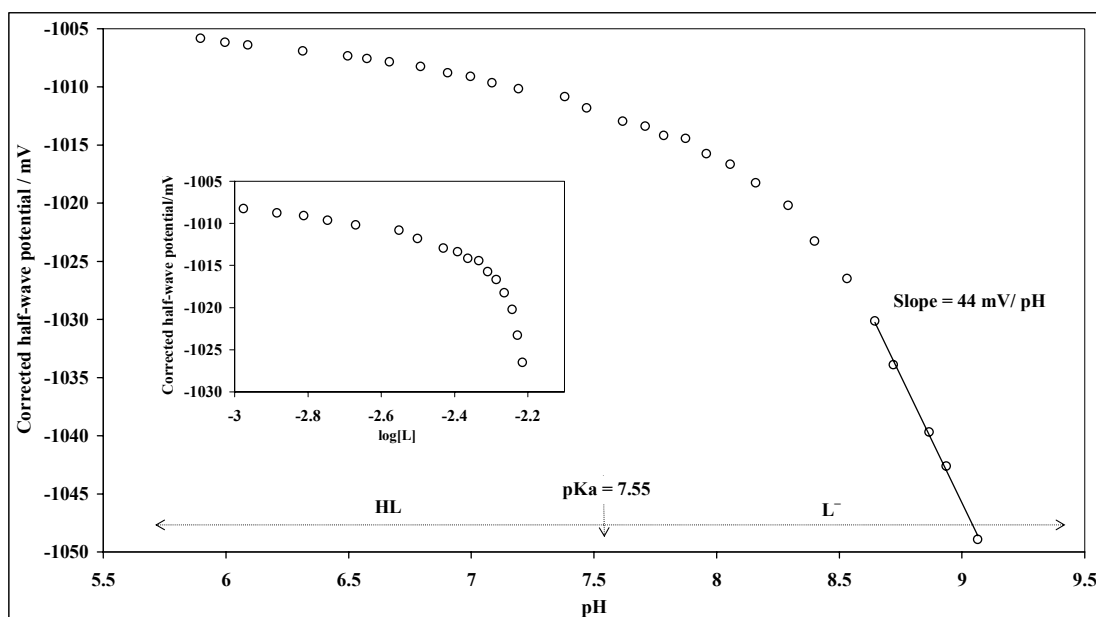
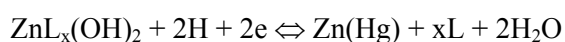
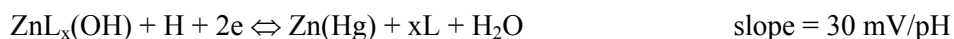


Figure 7-5 Corrected half-wave potential as a function of pH and as a function of the free ligand concentration (insert in this figure) obtained by DCP for $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ system. $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 255$, $[\text{Zn}^{2+}] = 2.8 \times 10^{-5} \text{ M}$.

The function shows a continuous and rather small shift of the half-wave potential towards cathodic potentials. Note that the total shift was around 45 mV. A slope of about 44 mV/pH, between pH 8.5 and 9.0, was the only one, which was possible to identify. This slope suggests the presence in solution of $\text{ZnL}_x(\text{OH})_y$ species. Reaction at the DME electrode with two protons involved should develop a slope of 59 mV/pH unit (charges omitted for sake of simplicity):



The fact that the slope did not develop to its theoretical value indicates the presence of some amount of $\text{ZnL}_x(\text{OH})$ (one proton implicated):



and most likely still the reduction of the complex ZnL (no proton involved):



causing the observed slope having a resultant value of several electrochemical processes taking place at the working electrode simultaneously.

The analysis of the variation of the corrected half-wave potential with the concentration of free ligand (insert in Figure 7-5), which should allow us to predict ZnL_n species, does not indicate in an obvious way the complexation between zinc and TAPSO (there is not a slope of about 30 mV/log [L] as expected for ZnL).

It is very important to emphasize that complexation occurs in the pH range, where the ligand is being deprotonated, becoming the main form of the ligand present in solution (Figure 7-5). This means that the slope analysis is difficult, since no theoretical slope will appear in the region where the deprotonation occurs considering the absence of hydroxide species ($\text{ZnL}_x + 2\text{e} \rightleftharpoons \text{Zn}(\text{Hg}) + x\text{L}$, slope = 0 mV/pH). Note that up to pK_a 7.55, only 8.6 mV shift was observed, indicating that the highest amount of complexed species should exist at pH values higher than the pK_a . A similar behaviour was observed for $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ system (Figure 7-2A). Note that for both systems, the ML complex starts to be formed in the deprotonation pH range, indicating that the highest amount of complexed species exist after the pK_a , which complicates the system characterization.

From the analysis of data seen in Figure 7-5 one could conclude that the species $\text{ZnL}_x(\text{OH})_y$ were detected and ZnL was expected to be formed to some extent. Assuming simplest model one should consider ZnL , $\text{ZnL}(\text{OH})$ and $\text{ZnL}(\text{OH})_2$.

Next, several calculated complex formation curves were generated to help in the decision on the final model of this system (Figure 7-6). The CCFC for only $\text{Zn}_x(\text{OH})_y$ species (dashed line curve) evidenced a lower shift than the ECFC. This was an unequivocal indication of complexation, even though the difference between the two curves at pH 8.0 (where the metal hydroxides species start to form) was only about 12 mV. Similar to what was observed with $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ system, the CCFC for metal-hydroxide species was parallel to the ECFC (open circles in Figure 7-6 at highest pH values), which means that $\text{ZnL}_x(\text{OH})_y$ and $\text{Zn}(\text{OH})_y$ species exist in the same pH range. When we compare the CCFC (dotted line curve) calculated only for hydroxide species ($\text{ZnL}(\text{OH})$, $\text{ZnL}(\text{OH})_2$ and $\text{Zn}_x(\text{OH})_y$) with the experimental data (circles), this clearly shows that this model does not describe the system. From pH 6.0 to 8.0, the CCFC is below the ECFC, which suggests the formation of another species prior to the formation of hydroxo species; clearly, the complex ZnL must be

included. Above pH 8.0 the inclusion of only hydroxide species in the M–L model makes the CCFC approaching the ECFC, but still does not describe totally the experimental data. The refined stability constants are summarized in Table 7-3.

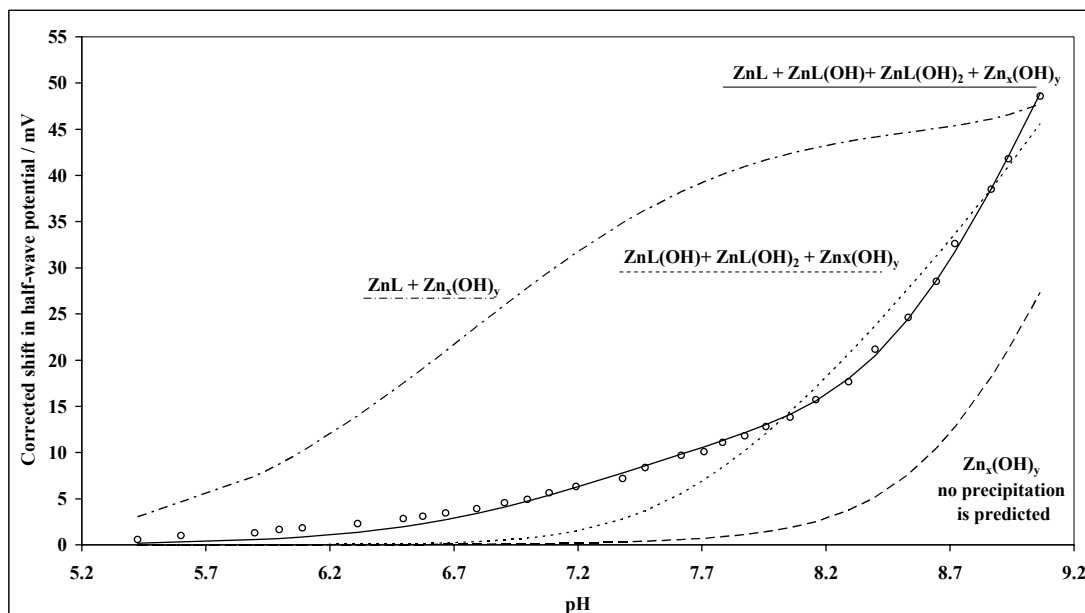


Figure 7-6 Experimental (points, DCP data) and calculated (lines) complex formation curves for the $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ system. $[\text{L}_\text{T}]:[\text{Zn}_\text{T}]$ ratio 255, $[\text{Zn}^{2+}] = 2.8 \times 10^{-5}$ M. The stability constant values are shown in Table 7-3.

The refined value for $\text{ZnL}(\text{OH})$ stability constant is very high when the complex ZnL is not included in the M–L model; if one considers the hydrolyses of the ZnL complex, $\log \beta_{\text{ZnL}} + \log \beta_{\text{Zn}(\text{OH})} = \log \beta_{\text{ZnL}(\text{OH})}$, this would result with a value for $\log \beta_{\text{ZnL}}$ of 3.5. This value is too high for Zn -TAPSO complexation. For the same ligand, we should expect that Pb^{2+} forms stronger complexes with TAPSO than zinc [14], but the computed value was of the same order of magnitude (Part III, Chapter 5). The refined stability constant for $\text{ZnL}(\text{OH})_2$ was close to the theoretically expected value. In addition, the overall fit was rather large, indicating that this model was not correct. In the next step, we decided to include the ZnL complex in the model (Table 7-3, Model 2, and Figure 7-6), as some indication of its presence was suggested in Figure 7-5 and since this was the only reasonable complex to be considered here. This improved significantly the overall fit (Table 7-3, Model 2) and the CCFC fitted very well the experimental points (solid line in Figure 7-6).

For triethanolamine, stability constants for ZnL and ZnL_2 are reported in the literature [11]. However, for $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ system, no experimental evidence of the presence of ZnL_2 was observed (Figure 7-5). When the model containing ZnL , ZnL_2 and $\text{ZnL}(\text{OH})_2$ was allowed to be refined, the complex ZnL_2 was systematically rejected (data not shown).

Table 7-3 Overall stability constants (as $\log \beta$ values) for $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ system, determined at 25 °C and $\mu = 0.1 \text{ M}$ by DCP for $[\text{L}_T]:[\text{Zn}_T] = 150$, $[\text{Zn}^{2+}] = 5.2 \times 10^{-5} \text{ M}$ (26 points, from pH 4.9 to 9.0) and for $[\text{L}_T]:[\text{Zn}_T] = 255$, $[\text{Zn}^{2+}] = 2.8 \times 10^{-5} \text{ M}$. (30 points, between pH 5.4 and 9.1) and by GEP for $[\text{L}_T]:[\text{Zn}_T] = 3.3$, $[\text{Zn}^{2+}] = 7.6 \times 10^{-4} \text{ M}$ (two titrations, 84 points, between pH 4.5 and 7.7).

Complexes	Equilibrium	DCP $[\text{L}_T]:[\text{Zn}_T] = 255$		DCP $[\text{L}_T]:[\text{Zn}_T] = 150$		GEP $[\text{L}_T]:[\text{Zn}_T] = 3.3$
		Model 1	Model 2	Model 1	Model 2	
ZnL^+	$\text{Zn}^{2+} + \text{L}^- \rightleftharpoons \text{ZnL}^+$	NI	2.45 ± 0.03	NI	2.47 ± 0.03	$2.63 \pm 0.02^*$
$\text{ZnL}(\text{OH})$	$\text{Zn}^{2+} + \text{L}^- + \text{OH}^- \rightleftharpoons \text{ZnL}(\text{OH})$	8.29 ± 0.02	7.2 ± 0.4	8.30 ± 0.02	7.2 ± 0.4	NI
$\text{ZnL}(\text{OH})_2^-$	$\text{Zn}^{2+} + \text{L}^- + 2\text{OH}^- \rightleftharpoons \text{ZnL}(\text{OH})_2^-$	12.0 ± 0.5	13.14 ± 0.05	12.95 ± 0.06	13.27 ± 0.03	NI
SD / mV		8.7	0.29	10.2	0.75	
Hamilton R-factor						0.030

For DCP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (Part II, Chapter 2, Section 2.3.3) For GEP, Hamilton R-factor stands for a statistical Hamilton R-factor generated by the program ESTA (Part V, Chapter 10, Section 10.2.4.1

Using the stability constant values refined from DCP experiments, a glass electrode potentiometry experiment was designed. Due to the early precipitation, around pH 7.8, only stability constant for ZnL was possible to refine (Table 7-3). This system showed to be very sensitive to the acid concentration and simultaneous refinement of this parameter had to be done in order to obtain reasonable results. Only the results, for which the variation of this parameter were lower than 2 % were used. The refined results for data collected between pH 4.5 and 7.7 from two titrations are summarized in Table 7-3. Similar results were obtained with the other titrations.

Considering potentiometric and polarographic experiments, the final model for Zn-(TAPSO)_x-(OH)_y system is: ZnL, ZnL(OH) and ZnL(OH)₂, with stability constants set to (as log β values): 2.5 ± 0.1 , 7.2 ± 0.4 and 13.2 ± 0.1 , respectively. Using these values, SDD for [L_T]:[Zn_T] ratios 255 and 3.3 were plotted (Figure 7-7). Observing the diagrams, it is clear why the stability constant value for ZnL(OH) has a large standard deviation, it exists in a very small amount for both ratios (Figure 7-7). For [L_T]:[Zn_T] ratio 255, at pH 8.5, where ZnL(OH) exists in higher amount, there is only 8 % of ZnL(OH), while 29 % of ZnL and 40 % of ZnL(OH)₂ are predicted in solution (Figure 7-7A). Therefore, it is understandable that the standard deviation associated with this constant would be large and for evident reasons, this species practically does not influence the system characterization. ZnL and ZnL(OH)₂ complexes are the most important species, which will influence the free zinc ion in the buffering pH region. Now, the absence of slopes in the polarographic graphical analysis (Figure 7-5) is also clear. ZnL is formed exactly in the deprotonation region, and even though 53 % exists at pH = pK_a (Figure 7-7A), the observed shift was only 8.6 mV (Figure 7-5). Figure 7-7B also evidences that, for GEP experimental conditions, ZnL was the only species formed before precipitation was predicted and this was just what was observed experimentally (precipitation was detected around pH 7.8) (Figure 7-7B).

When we compare the final models obtained for Cd-(TAPSO)_x-(OH)_y and Zn-(TAPSO)_x-(OH)_y systems, the main difference is that, for Cd-(TAPSO)_x-(OH)_y system, a large amount of CdL₂ is formed in a wide range of pH (between pH 7.5 and 9.0) for higher [L_T]:[Cd_T] ratios, whereas for Zn-(TAPSO)_x-(OH)_y system in the same pH range, no experimental evidence of ZnL₂ was detected and ZnL is the main species followed by an exponential increase of ZnL(OH)₂. This fact can be easily understood. First, the stability constant of ZnL is slightly higher than the stability constant determined for CdL. In addition, the stability constant value for Zn(OH)₂ is more than two orders of magnitude larger than that for Cd(OH)₂ (Table 7-1), which favours the formation of ZnL(OH)₂ instead of ZnL₂.

Literature describes stability constants for ZnL of the same order of magnitude for other similar β -alcohols-amino ligands (2.27 and 2.38 for 2-amino-2-hydroxymethyl-1,3-propanediol (THAM or TRIS) and 2-bis(2-hydroxyethyl)amino-2-hydroxymethyl-1,3-propanediol (BIS-TRIS) respectively) [11].

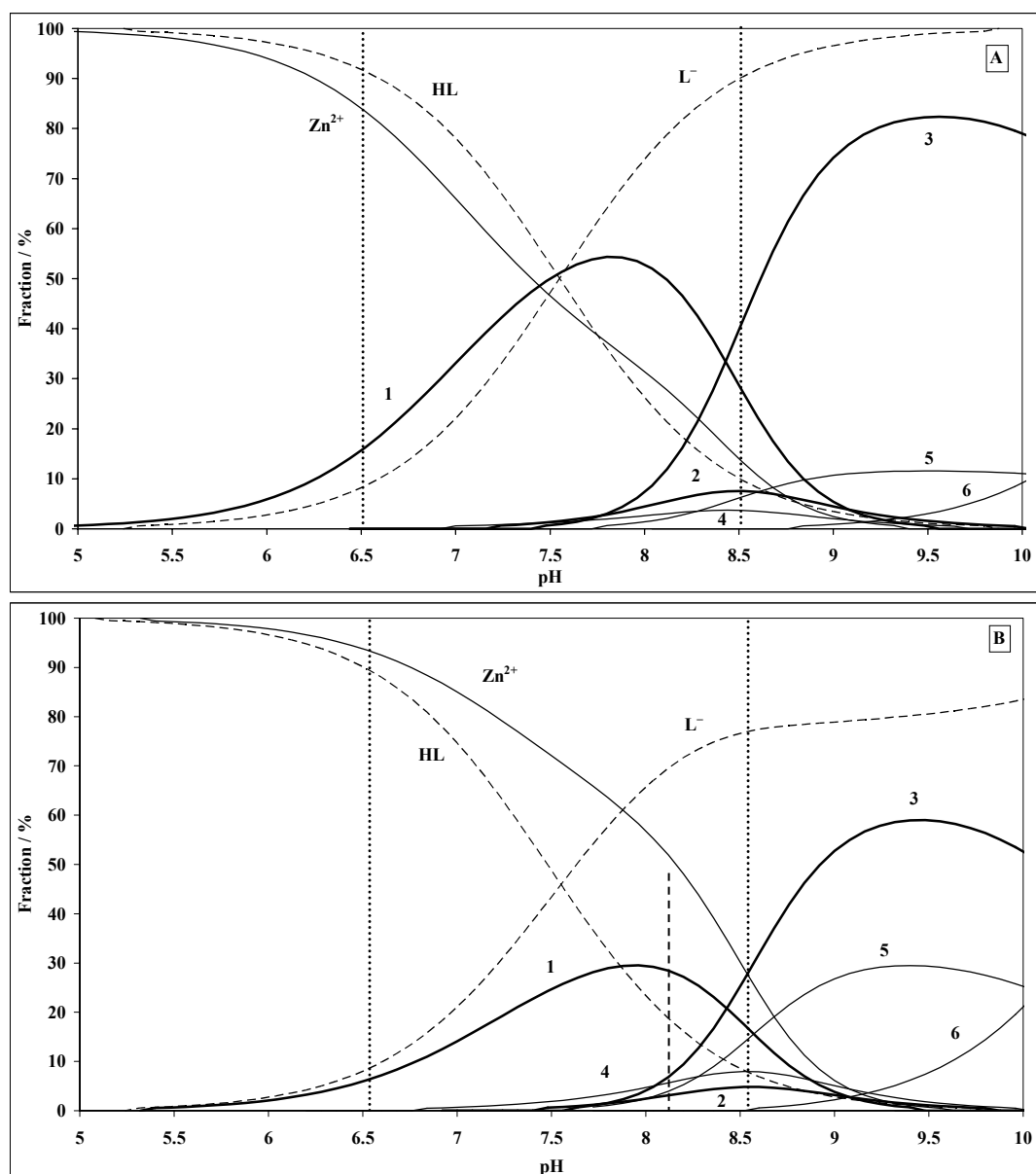


Figure 7-7 Species distribution diagrams for the Zn-(TAPSO)_x-(OH)_y system. (A) [L_T]:[Zn_T] ratio 255, [Zn²⁺] = 2.8×10^{-5} M; (B) [L_T]:[Zn_T] = 3.3, [Zn²⁺] = 7.6×10^{-4} M. Stability constants were set to (as log β values): ZnL = 2.5 (1), ZnL(OH) = 7.2 (2) and ZnL(OH)₂ = 13.2 (3). Zn_x(OH)_y species were also considered (Table 7-1): Zn(OH) (4), Zn(OH)₂ (5) and Zn(OH)₃ (6). Dashed line represents Zn(OH)₂ precipitation and the pH range bounded by the dotted lines indicates the pH buffering region.

On the other hand, some recent studies [4-6] have described stability constants values for ZnL (log β between 3.60 and 3.80) significantly different from the value obtained in this work. It has been decided to generate the CCFC for this model, ZnL plus Zn_x(OH)_y species, as reported in the literature (an average value of all published values for log β_{ZnL} was used, log β_{ZnL} = 3.70, see line -.- in Figure 7-6). The CCFC clearly shows that the value of stability constant is too high and does not describe the experimental values (ECFC).

8 COMPLEX FORMATION IN THE REGION OF METAL HYDROLYSIS AND M(OH)₂ PRECIPITATION. A GLASS ELECTRODE POTENTIOMETRIC AND POLAROGRAPHIC STUDY OF Cd-(AMPSO)_x-(OH)_y AND Zn-(AMPSO)_x-(OH)_y SYSTEMS⁷

ABSTRACT

The interaction between cadmium or zinc and AMPSO was investigated by DCP and GEP, at fixed total ligand to total metal concentration ratios and various pH values, at 25.0 °C and 0.1 M KNO₃ ionic strength. For Cd-(AMPSO)_x-(OH)_y system, CdL and CdL(OH) species, were identified, with stability constants values set to (as log β): 2.1 ± 0.1 and 6.2 ± 0.2 , respectively. For Zn-(AMPSO)_x-(OH)_y system, the proposed final model with stability constants set to (as log β) is: $ZnL = 2.5 \pm 0.1$ and $ZnL(OH)_2 = 12.9 \pm 0.2$. For both systems, the fact that AMPSO deprotonation occurs in the metal hydrolysis and M(OH)₂ precipitation and the complexes formed are not too strong added a real challenge to data interpretation.

8.1 INTRODUCTION

The compound AMPSO belongs to a broad series of biological buffers, which are commercially available; Sigma-Aldrich devotes a special section of its catalogue to these compounds. These buffers are zwitterionic N-substituted aminosulfonic acids containing hydroxyl groups on the β carbons. Thus, they are potential complexing agents.

Studies involving metal ions (such as is the case of heavy metals speciation, biological and biochemical studies), which require pH control, are inevitable subject to the possibility of buffer interference as a result of metal complexation. So, when these buffers are used in studies involving metals ions in solution, it is very important to consider the possible interference of them, due to metal ion complexation. If complexes are formed, then stability constants must be known and considered on the speciation simulations.

In a previous study, the interaction between AMPSO and copper was studied (Part II, Chapter 3). That study demonstrated that AMPSO binds copper strongly; a decrease between four and ten orders of magnitude in the free copper concentration in the pH-range (pH 8.0 and 10.0), where AMPSO is

⁷ Electroanalysis (2006) accepted for publication

efficient as a buffer, was predicted in the presence of 7.5×10^{-3} M of AMPSO and 4.9×10^{-5} M of copper. These results can be regarded as a huge change in the free copper concentration.

As far as we know, no reports regarding studies of the interaction between AMPSO and cadmium are described in the literature. For zinc, an exhaustive speciation study of $\text{Zn}-(\text{AMPSO})_x-(\text{OH})_y$ system has never been performed and the reported formation constants [15] between zinc and AMPSO do not meet the critical selection defined by Martell and Smith [16, 17]. Therefore, we think that the study of the interaction of AMPSO with cadmium and zinc may furnish a further contribution to obtain a systematic information of the overall stability constants of the $(\text{AMPSO})-(\text{metal ion})$ systems where complexation occurs.

In this work, the behaviour of AMPSO and cadmium or zinc was studied by two electrochemical techniques: DCP and GEP, at fixed total ligand to total metal concentration ratios and various pH values, at 25.0 °C and 0.1 M KNO_3 ionic strength. The final metal–ligand models for the two systems were properly defined by DCP. The quasi-reversibility of the electrochemical reduction of zinc ion was corrected using a procedure already employed successfully in another system (Part II, Chapter 3, Section 3.3.1). ACP was run in the absence of the metal ion in order to evaluate the adsorption of the ligand on the surface of the working mercury electrode.

8.2 EXPERIMENTAL

8.2.1 MATERIALS AND REAGENTS

A detailed description of the materials and reagents were reported previously (Part II: Chapter 2, Section 2.2.1, Chapter 3, Section 3.2.1 and Chapter 7, Section 7.2.1).

8.2.2 APPARATUS

All measurements reported in this work were performed on solutions adjusted to ionic strength 0.1 M KNO_3 in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer, and thermostatted at 25.0 ± 0.1 °C using a water bath.

8.2.2.1 POLAROGRAPHY

The polarographic measurements of $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ and $\text{Zn}-(\text{AMPSO})_x-(\text{OH})_y$ systems, as

well as alternating current studies, were performed as previously described (Part II, Chapter 4, Section 4.2.2.1).

8.2.2.2 POTENTIOMETRY

Potentiometric titrations of Cd-(AMPSO)_x-(OH)_y and Zn-(AMPSO)_x-(OH)_y systems were performed as described previously (Part II, Chapter 2, Section 2.2.2.2).

8.2.3 PROCEDURE

8.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

For all techniques described bellow, the calibration of the glass electrodes (measurements of pH as $-\log [H^+]$) was achieved as previously described (Part II, Chapter 3, Section 3.2.3.1).

8.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

In order to evaluate the adsorption of the ligand, AMPSO, on the surface of the mercury working electrode, AC polarograms were run in the absence of the metal ion. The experimental conditions used were described elsewhere (Part II, Chapter 3, Section 3.2.3.2).

The polarograms were scanned in the potential range between 0.2 and -1.2 V *versus* Ag/AgCl(s), 3 M KCl at three different pH values (8.0, 9.0 and 10.0), varying the ligand concentrations from 4.0×10^{-4} to 1.3×10^{-2} M.

8.2.3.3 DIRECT CURRENT POLAROGRAPHY

DC polarographic titrations were performed, for both systems Cd-(AMPSO)_x-(OH)_y and Zn-(AMPSO)_x-(OH)_y, at fixed total ligand to total metal ion concentration ratios ($[L_T]:[M_T]$) and varied pH. For Cd-(AMPSO)_x-(OH)_y system, two different $[L_T]:[Cd_T]$ ratios were examined: 357 and 476 with a total cadmium concentration of 4.2×10^{-5} M. A drop time of 2 s and a step potential of 4 mV were used. For Zn-(AMPSO)_x-(OH)_y system, $[L_T]:[Zn_T]$ ratios equal to 609 and 799 were investigated with a total zinc concentration of 2.5×10^{-5} M. A drop time of 1 s and a step potential of 4 mV were used. The procedure used to run the polarographic titrations was described elsewhere (Part II, Chapter

3, Section 3.2.3.4); a set of 30 and 40 polarograms (representing all the species present at each pH value) were recorded in the pH range between 7.1 and 9.5 (no further points could be collected because precipitation occurred at pH 9.5) for Cd-(AMPSO)_x-(OH)_y system and in the pH range between 6.2 and 10.6 for Zn-(AMPSO)_x-(OH)_y system. The equilibrium of the metal-ligand solutions was tested and it was reached in a few minutes.

8.2.3.4 GLASS ELECTRODE POTENTIOMETRY

For Cd-(AMPSO)_x-(OH)_y system, four titrations, using two independent solutions, were performed in the pH range between 6.2 and 8.0, using dynamic and monotonic titrant volume additions; between 25 and 40 points were collected for each type of titration. The total cadmium concentration was kept at about 3.0×10^{-3} M and a [L_T]:[Cd_T] ratio of 5.0 was used. For Zn-(AMPSO)_x-(OH)_y system, a [L_T]:[Zn_T] ratio of 25.0 was studied with a total zinc concentration of 1.0×10^{-3} M. Four titrations, using two independent solutions, were performed in the pH range between 6.0 and 7.8, using monotonic standardized KOH (0.1M) additions. For each titration, about 40 points were collected. For both metal-ligand systems studied, points were collected until pH 10.0 for the first titration in order to confirm when precipitation occurs.

8.2.4 DATA TREATMENT

The simulation and optimization procedures of potentiometric data was done using ESTA program [7, 8]; for more details about the program see Part II, Chapter 2, Section 2.3.3 and Part V, Chapter 10, Section 10.2.4.1.

The refinement of polarographic data was performed using the Cukrowski method [9, 10]; as it was described previously (Part II, Chapter 2, Section 2.2.4).

During the refinement of the overall stability constants for both systems, the water [11] (Part II, Chapter 2, Table 2-1) and ligand (Part II, Chapter 3, Table 3-1) protonation constants, as well as all known stability constants for Cd_x(OH)_y and Zn_x(OH)_y species [11] (Table 7-1), were kept fixed.

8.3 RESULTS AND DISCUSSION

8.3.1 LIGAND ADSORPTION AND METALS REVERSIBILITY STUDIES

The possible adsorption of AMPSO at the mercury electrode was studied by ACP because stability constants obtained by polarographic techniques can be significantly influenced by the ligand and/or complexes adsorption; the best experimental conditions would be in the absence of adsorption of the ligand/complexes. The curves of alternating current *vs.* applied potential were recorded between 0.2 and -1.2 V with a phase angle of 90°. The adsorption studies were carried out at three pH values (8.0, 9.0 and 10.0) for evaluating the extent of adsorption of the different ligand forms. The pH values were chosen taking into account the different forms of the monoprotonated ligand; at pH 8.0, the ligand exists mainly in its protonated form, HL, at pH 10.0, it exists predominantly in the deprotonated form, L⁻, and at pH equal to p*K*_a value, it exists half in each form.

By comparing the AC polarograms between background solution and the polarograms obtained with increasing AMPSO additions, the adsorption effect can be quantified. In Figure 8-1, the variation of the capacitive current obtained with the increase of the AMPSO concentration relative to the background solution (0.1 M KNO₃), expressed in percentage, is represented for pH 9.0. Figure 8-1 shows that, in the potential region where cadmium and zinc are reduced (-0.54 and -0.96 V, respectively [18]), the decrease of the capacitive current was insignificant (less than 6%) in the concentration range studied ([AMPSO] < 1.3 × 10⁻² M). For the other pH values studied, the interaction was lower (data not shown).

The polarograms recorded for Cd-(AMPSO)_x-(OH)_y system indicated that the electrochemical process was fully reversible. For both polarographic titrations, fully reversible DC polarograms were recorded (the polarographic wave steepness varied between 0.96 and 1.0).

The zinc reduction process at the mercury electrode surface was not fully reversible. A decrease in the DC reversibility was observed. For [L_T]:[Zn_T] = 799, from pH 6.2 up to pH 10.6, the steepness of the polarographic waves decreased from 0.9 to 0.4; for [L_T]:[Zn_T] = 609, the wave steepness decreased from 1.0 (pH ≈ 6.8) to 0.8 (pH ≈ 9.6). These results evidenced an increase of the irreversibility of the reduction process for higher pH values. In order to use the collected data, and since the limiting diffusion current *I*_d does not depend on the degree of the reversibility of the electrochemical process, a correction of the irreversibility already used successfully in another system was applied. However, it was not possible to correct the irreversibility for wave steepness values below 0.8. The procedure employed to correct the DCP data was described in a previous work (Part II, Chapter 3, Section 3.3.1).

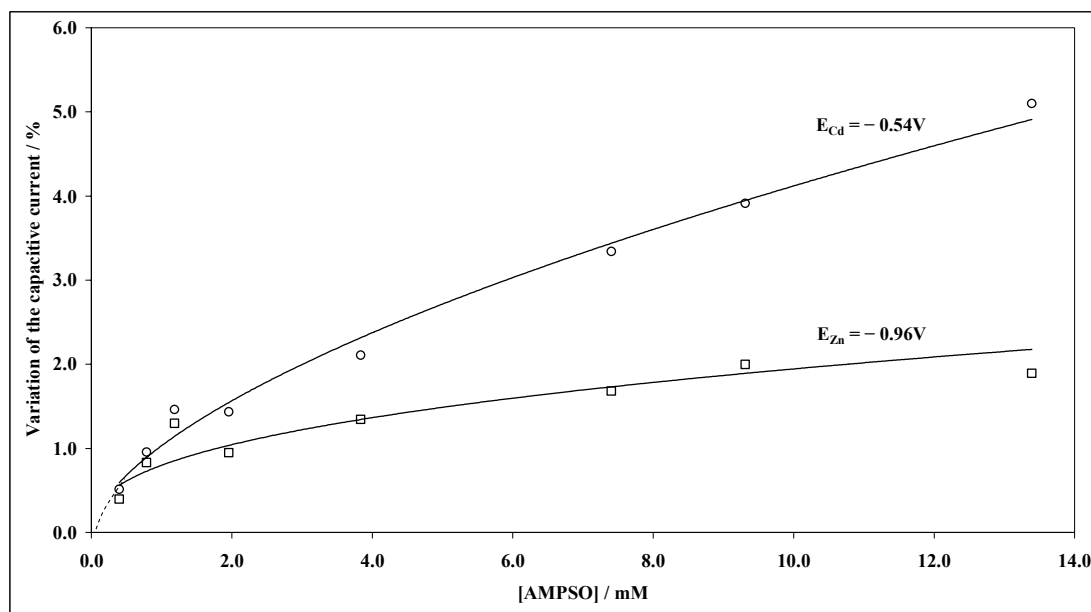


Figure 8-1 Variation of the capacitive current recorded by ACP, without metal ions, with the increase of AMPSO concentration, at pH 9.0. The values, expressed in percentage, were calculated from the difference between capacitive current values recorded in the absence and in the presence of AMPSO, at a potential of -0.54 or -0.96 V. ACP curves were recorded with a phase angle of 90° in 0.1 M KNO_3 .

8.3.2 MODELLING AND REFINEMENT OF STABILITY CONSTANTS

The analysis of DC polarographic titrations in a pH range between 6.2 and 9.5 using different ligand to metal ratios showed that $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ and $\text{Zn}-(\text{AMPSO})_x-(\text{OH})_y$ systems behaved as labile on the polarographic time scale; only one wave corresponding to all labile species present in solution, which shifted to more cathodic potential values, was observed (data not shown).

The choice of experimental conditions to characterize $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ and $\text{Zn}-(\text{AMPSO})_x-(\text{OH})_y$ systems was very complicated. First, AMPSO protonation constant is very basic ($\text{p}K_a = 9.05$). In addition, AMPSO is structurally related with other biological buffers (TAPSO, TRIS and BIS-TRIS), which do not form strong complexes with cadmium and zinc ([11], Part II, Chapter 7). Due to that, we did not expect that AMPSO bound strongly with cadmium and zinc.

The combination of potentiometry and polarography allowed us to cover a wide range of total metal concentration (between 10^{-5} and 10^{-3} M) and a wide range of $[\text{L}_T]:[\text{M}_T]$ ratios (between 5 (cadmium) or 25 (zinc), by GEP, and 357 (cadmium) or 799 (zinc), by polarography). As far as we know, no other combination of techniques should be able to cover a so wide range of total metal concentration and $[\text{L}_T]:[\text{M}_T]$ ratios with these type of ligands. It is important to note that AMPSO is a very simple ligand with no double bonds. Therefore UV-Vis and fluorescence, which are the only techniques that use low concentration of ligands (between 10^{-7} and 10^{-4} M), cannot be applied for

these systems because neither the metal ions nor AMPSO absorb. In addition, by GEP, neither lower nor higher [L_T]:[M_T] ratios were possible to run. For lower [L_T]:[M_T] ratios, both metals (cadmium or zinc) systems would have earlier precipitation of M(OH)₂ and no further information would be obtained. Because complexation starts in the deprotonation range of the ligand, higher [L_T]:[M_T] ratios were not possible to run because all the titration goes towards the deprotonation of the ligand and not to neutralise protons liberated from the metal complexation (for example: M + HL = ML + H).

8.3.2.1 Cd-(AMPSO)_x-(OH)_y SYSTEM

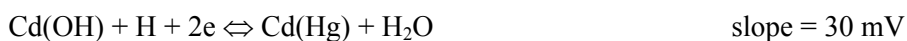
By DC polarography, two [L_T]:[Cd_T] ratios were carried out: [L_T]:[Cd_T] = 357 and 476, with [Cd²⁺] = 4.2 × 10⁻⁵ M. Figure 8-2A shows the variation of the half-wave potential shift as a function of the pH and the logarithm of the free AMPSO concentration (insert of the figure) for the lowest ratio. Protonation constant for the ligand and the different forms of the ligand according to the pH values are also marked in the figure. The total potential shift recorded was only 12.7 mV. It is fundamental to emphasize that it corresponds to the pH range where the deprotonation of the ligand occurs, which means that the two forms of the ligand are present in solution, causing a small variation in the slopes (as it will be discussed below) and additionally in the total potential shift. Three different regions were identified with the variation of pH. In the first region – I, up to pH ≈ 8.3, an incipient complexation was obtained; a shift of less than 2 mV was observed. In region II, a slope of about 7 mV / pH was observed. This slope is smaller than one would expect for any metal containing species of ML type. Theoretical slope for CdL (one proton in the electrochemical reaction), would be (charges were omitted for sake of simplicity):



However, between pH 8.0 and 10.0 the ligand exists in the two forms, protonated and deprotonated, which means that the reaction with no protons involved, must also be considered:



This is why the slope never develops to its theoretical value. So, CdL must be formed even though the slope does not suggest that. Next in region III, above pH 9.0, the slope increased to 13 mV / pH. This increase can be due to the formation of Cd(OH) and/or CdL_x(OH), since the reduction of these species at the DME electrode, would generate a theoretical slope of about 30 mV / pH, only one proton involved:





$$\text{slope} = 30 \text{ mV}$$

The fact that the obtained slope did not reach this value can be rationalized by the simultaneous formation of CdL . After the pK_a , the ligand is mainly in the deprotonated form, which means that the main reduction reaction of CdL , at the DME, is the second one described above (no proton involved). This caused the drop in the theoretical slope.

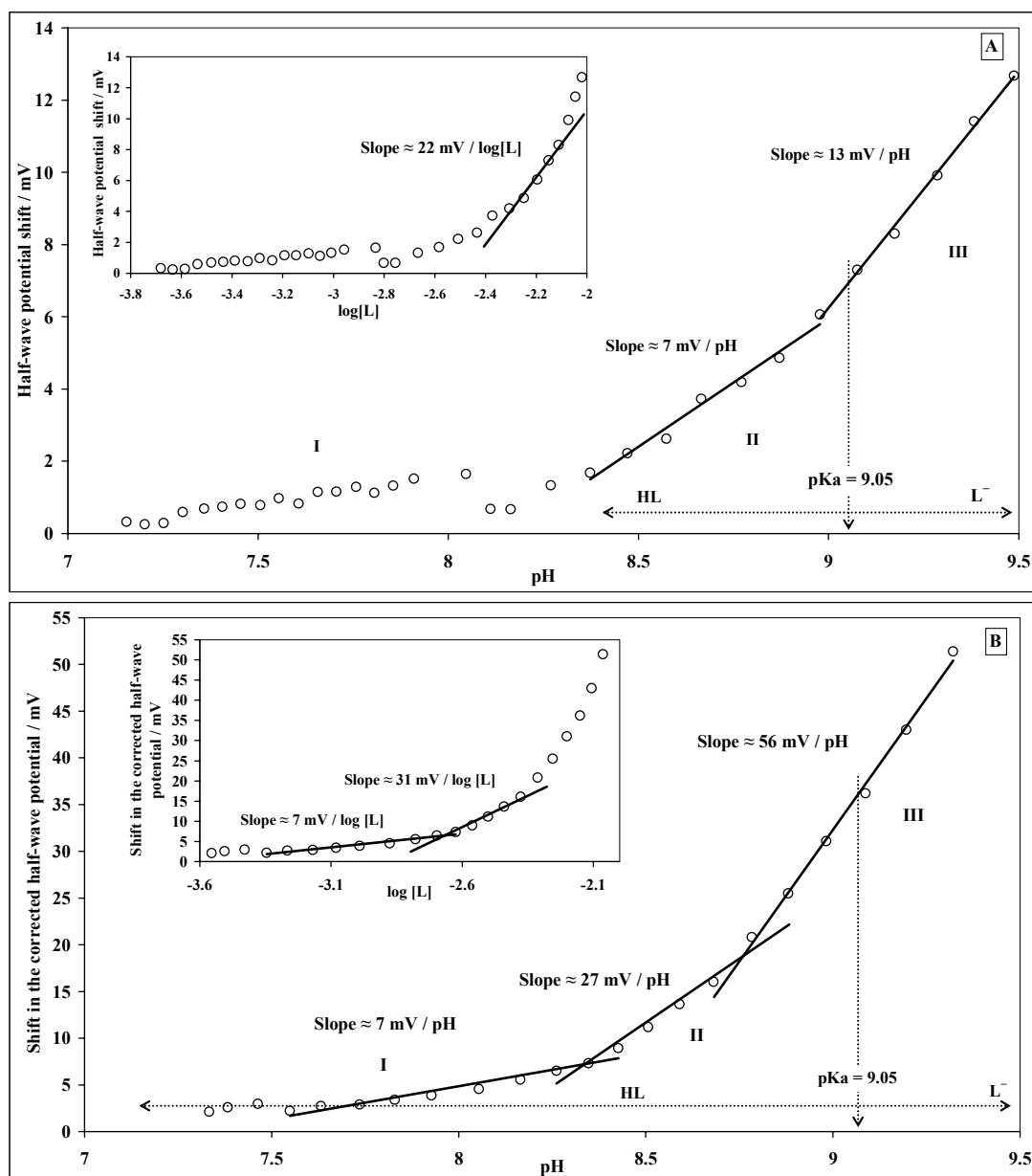


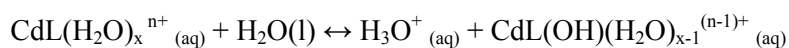
Figure 8-2 Half-wave potential shift as a function of pH and the free ligand concentration (insert), recorded by DCP, for (A) $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ system, $[\text{L}_T]:[\text{Cd}_T] = 357$ with $[\text{Cd}^{2+}] = 4.2 \times 10^{-5} \text{ M}$ and (B) $\text{Zn}-(\text{AMPSO})_x-(\text{OH})_y$ system, $[\text{L}_T]:[\text{Zn}_T] = 609$ with $[\text{Zn}^{2+}] = 2.5 \times 10^{-5} \text{ M}$. For both systems, experiments were run at 25°C and 0.1 M KNO_3 ionic strength.

The analysis of the half-wave potential shift versus log [L] (insert in Figure 8-2A) confirms the formation of CdL (slopes reaches a value of about 22 mV per log [L] unit) for the pH range between 8.7 and 9.2.

From the graphical analysis described above, the starting model for Cd-(AMPSO)_x-(OH)_y system should include CdL and CdL_x(OH) species.

The comparison between CCFC for Cd(OH)_x species alone (dashed line in Figure 8-3A) and ECFC (circles in Figure 8-3A) clearly shows that AMPSO complexes cadmium, since a higher cathodic shift of the potential was obtained in the presence of AMPSO when compared with the CCFC predicted for Cd(OH)_x species alone. In addition, precipitation of Cd(OH)₂, in the absence of AMPSO, was predicted around pH 8.9, and that was not confirmed experimentally; no precipitation was observed up to pH 9.5. The procedure used to obtain the final model was based on fitting the CCFC for several models to the ECFC (Figure 8-3A). The numerical results are summarized in Table 8-1.

Even though the graphical analysis in Figure 8-2 has suggested that CdL(OH) species would be probably present in solution, we decided to test a simpler model by fitting CdL plus Cd_x(OH)_y into the experimental data (Table 8-1, Model 1 and Figure 8-3A). However, when the stability constant for this species was refined and the CCFC drawn, it became obvious that CdL_x(OH) species must be formed. The graphical analysis of the fit revealed that the stability constant value for CdL was clearly too large (dotted line in Figure 8-3A). The CCFC for this model (dotted line, Figure 8-3A) was above the ECFC up to pH 9.08, which indicated that the stability constant value for CdL, the main species present in solution at this pH range, was too high. Above pH 9.08 the CCFC was below the ECFC, indicating that other species were missing. In Model 2, the inclusion of CdL(OH) species (Table 8-1, Model 2 and Figure 8-3A) resulted in a very good fitting with a low overall standard deviation. For [L_T]:[Cd_T] = 476, similar results were obtained (Table 8-1). The refined stability constant value for CdL(OH) species suggests that the formation of this species resulted from the hydrolysis of CdL, according to the following reaction:



since the value of the stability constant of the species CdL(OH) is just the sum of: $\log \beta_{\text{CdL}} + \log \beta_{\text{Cd}(\text{OH})} = \log \beta_{\text{CdL}(\text{OH})} = 2.0 + 4.3 = 6.3$.

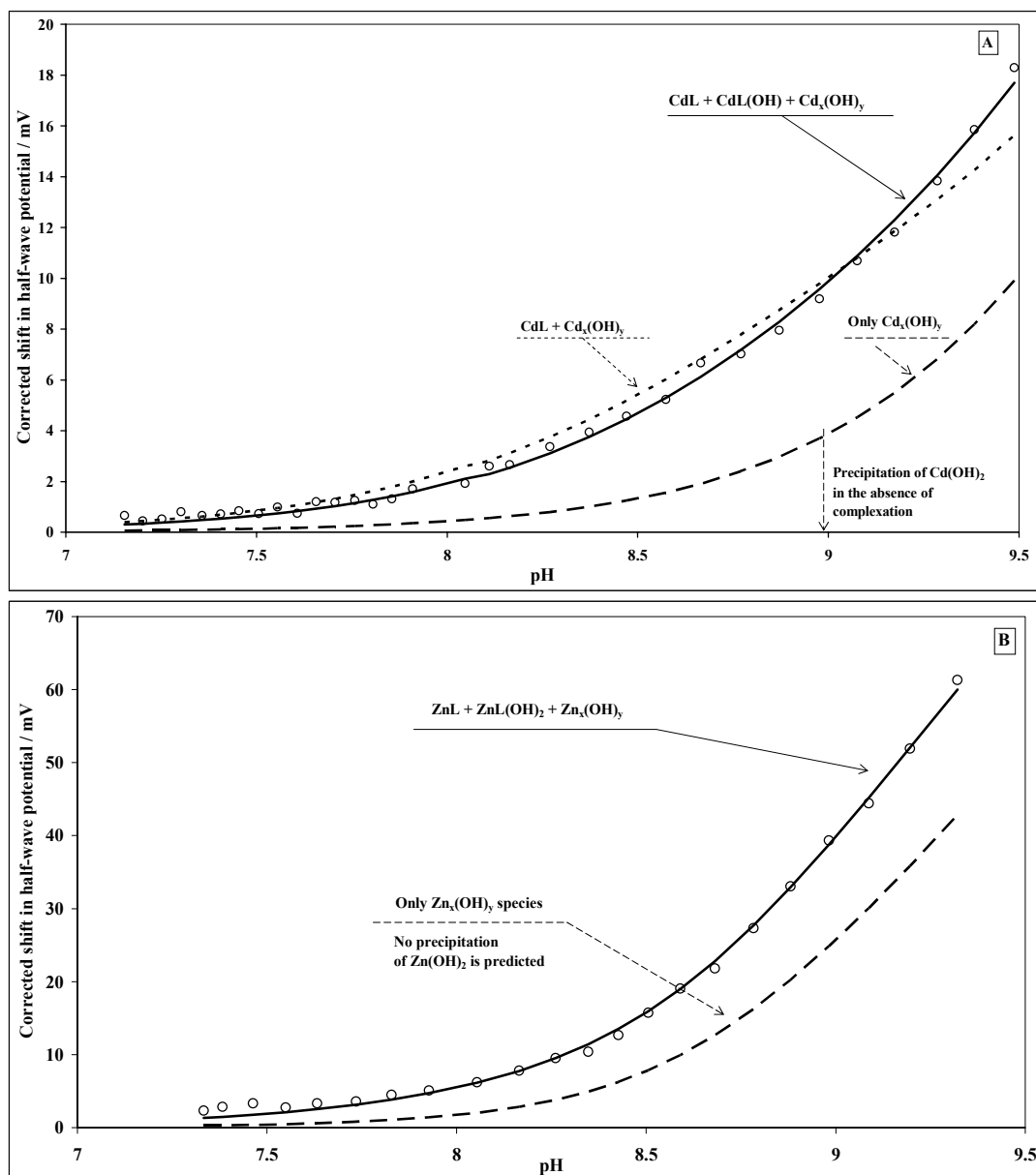


Figure 8-3 Experimental (circles) and calculated complex formation curves (lines) for DCP data: (A) Cd-(AMPSO)_x-(OH)_y system: $[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 357$, $[\text{Cd}^{2+}] = 4.2 \times 10^{-5}$ M; (B) Zn-(AMPSO)_x-(OH)_y system: $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 609$, $[\text{Zn}^{2+}] = 2.5 \times 10^{-5}$ M. Fitted models for cadmium and zinc are indicated in the figure and the refined stability constant values are summarized in Table 8-1 and Table 8-2, respectively.

Table 8-1 Overall stability constants for Cd-(AMPSO)_x-(OH)_y system determined at 25 °C and *I* = 0.1 M by DCP for [L_T]:[Cd_T] = 357 and [L_T]:[Cd_T] = 476, [Cd²⁺] = 4.2 × 10⁻⁵ M (32 points, between pH 7.1 and 9.5 and 27 points from pH 7.1 to 9.5, respectively) and GEP [L_T]:[Cd_T] = 5.0, [Cd²⁺] = 3.0 × 10⁻³ M (144 points, four titrations, from pH 6.2 to 8.0).

Complexes / Equilibrium	DCP [L _T]:[Cd _T]=357		DCP [L _T]:[Cd _T]=476		GEP [L _T]:[Cd _T]=5.0
	Model 1	Model 2	Model 1	Model 2	
CdL Cd + L ⇌ CdL	2.14 ± 0.03	2.00 ± 0.06	2.16 ± 0.02	2.07 ± 0.04	2.192 ± 0.002
CdL(OH) Cd + L + OH ⇌ CdL(OH)	NI	6.3 ± 0.1	NI	6.1 ± 0.1	NI
SD / mV	0.48	0.072	0.56	0.17	
Hamilton R-factor					0.0050

For DCP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (Part II, Chapter 2, Section 2.3.3). For GEP, Hamilton R-factor stands for a statistical Hamilton R-factor generated by the program ESTA (Part V, Chapter 10, Section 10.2.4.1). NI – not included.

In order to confirm the stability constants values obtained for Cd-(AMPSO)_x-(OH)_y system by polarography, predictions based in SDD, using Model 2 refined from polarography (Table 8-1), were made for designing the best analytical conditions for GEP experiments. The SDD (data not shown) suggested that it should be possible to establish CdL stability constant prior to precipitation but no information about CdL_x(OH)_y species would be available. Because complexation starts in the deprotonation range of the ligand and in order to minimise the titration contribution towards the deprotonation of the ligand instead of neutralisation of protons liberated from the cadmium complexation (for example: Cd + HL = CdL + H), experimental conditions were chosen only for [L_T]:[Cd_T] ratios, where the expected $[L] / \left[\sum_{x,y=0}^2 \text{CdL}_x(\text{OH})_y \right]$ ratio were lower than 5. GEP titrations using a total cadmium concentration of 3.0 × 10⁻³ M, with [L_T]:[Cd_T] = 5.0, were run. Precipitation was observed at pH ≈ 8.0. The refined results are summarized in Table 8-1. The stability constant value obtained for CdL was very comparable with the ones obtained by polarography.

Considering all the refined values obtained from the different ratios and the different techniques performed, the final model proposed is (stability constants as log β values): CdL = 2.1 ± 0.1 and CdL(OH) = 6.2 ± 0.2 (note that the standard deviations indicated are referred to the interval of variation of the values of the stability constants). Species distribution diagrams for the proposed final model for [L_T]:[Cd_T] = 357 and [L_T]:[Cd_T] = 5.0 ratios were plotted (Figure 8-4).

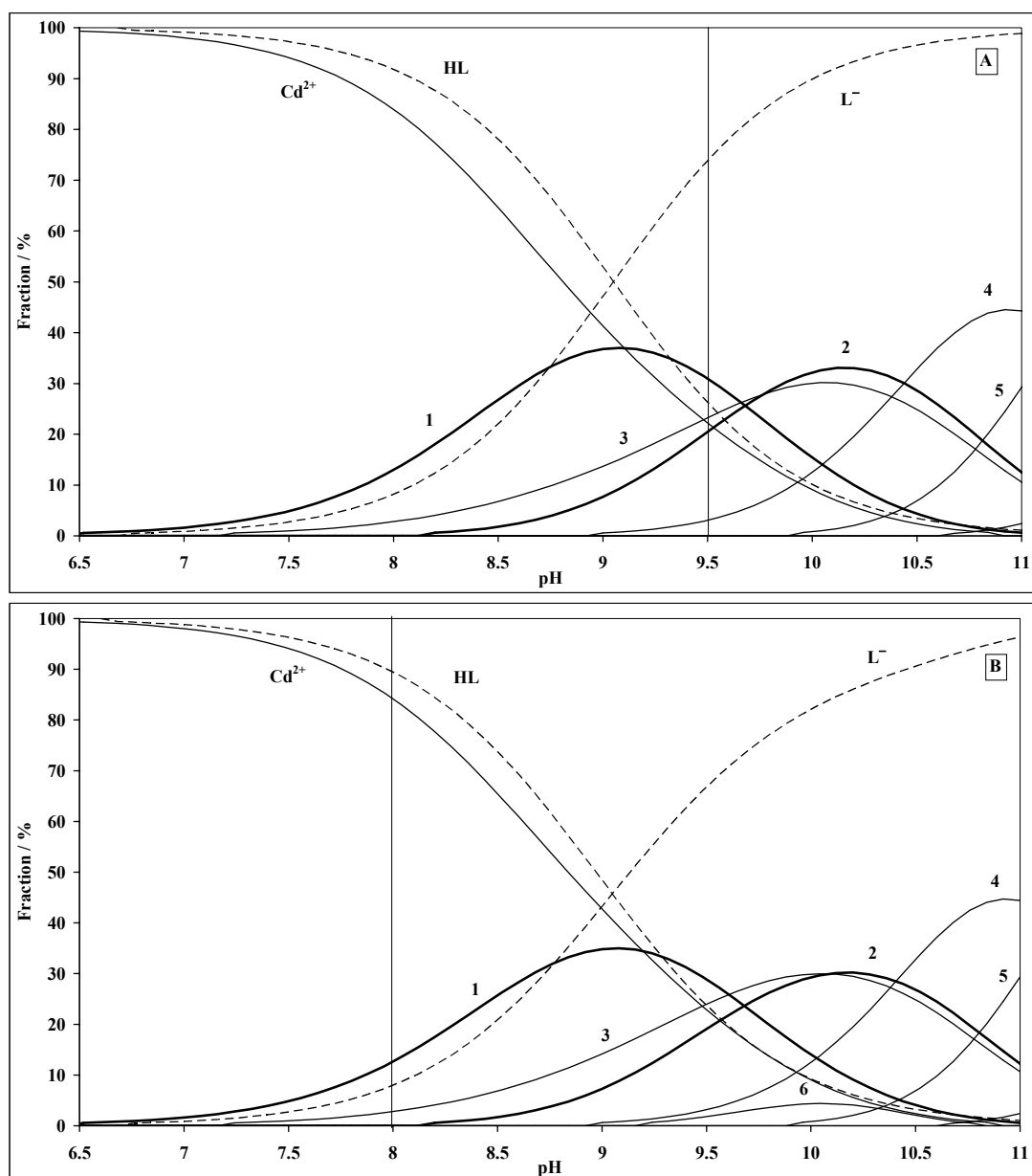


Figure 8-4 Species distribution diagrams computed for $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ system. (A) DCP experimental conditions ($[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 357$ with $[\text{Cd}^{2+}] = 4.2 \times 10^{-5} \text{ M}$); (B) GEP experimental conditions ($[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 5.0$ with $[\text{Cd}^{2+}] = 3.0 \times 10^{-3} \text{ M}$). The metal-ligand model consisted of CdL (1), $\text{CdL}(\text{OH})$ (2), $\text{Cd}(\text{OH})$ (3), $\text{Cd}(\text{OH})_2$ (4), $\text{Cd}(\text{OH})_3$ (5) and $\text{Cd}_4(\text{OH})_4$ (6), for which stability constants, as $\log \beta$, were set to 2.1, 6.2, 4.3, 7.7, 10.3 and 24.9, respectively. The vertical line indicates the pH at which precipitation was observed.

For the lower ratio (Figure 8-4B), the main species present in solution prior to precipitation is CdL , which is in agreement with the obtained results (Table 8-1). The species distribution diagram for $[\text{L}_\text{T}]:[\text{Cd}_\text{T}]$ ratio 357 (Figure 8-4A) shows that up to pH 9.5 the main species in solution are CdL , $\text{CdL}(\text{OH})$ and $\text{Cd}(\text{OH})$, which confirms the slopes identified in Figure 8-2A and explains the stability constants values for $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ system summarized in Table 8-1.

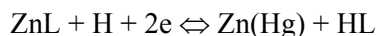
The results presented above show that, in the pH range where the buffer is used (between pH 8.0 and 10.0), besides the formation of Cd(OH)_y species, cadmium forms two complexes with AMPSO, CdL and CdL(OH) (Figure 8-4).

8.3.2.2 Zn-(AMPSO)_x-(OH)_y SYSTEM

For Zn-(AMPSO)_x-(OH)_y system, [L_T]:[Zn_T] ratios equal to 609 and 799 were investigated by DCP with a total zinc concentration of 2.5×10^{-5} M. In Figure 8-2B, the shift in the corrected half-wave potential as a function of pH and the logarithm of ligand concentration (insert of the figure) for [L_T]:[Zn_T] ratios equal to 609 is shown. The different forms of the ligand according to the pH and AMPSO's p*K*_a value are also marked in the figure. The function shows a continuous and slow shift of the potential. It is very important to emphasize that complexation occurs at high pH, in a region where the ligand is being deprotonated, becoming the main form of the ligand present in solution (Figure 8-2B). Three slopes can be identified: two before the protonation constant and one after. Between pH 7.5 and 8.2, region I, a slope of about 7 mV / pH was obtained. This slope is too low; it just indicates that complexation started. The same can be observed in the insert in Figure 8-2B. In region II, from pH 8.2 up to pH 8.8, the slope reached a value of 27 mV / pH. Above pH 8.8 until pH 9.3, region III, the slope was 56 mV / pH. This last slope clearly indicates the presence in solution of ZnL_x(OH)₂ species. The reduction reaction of the latter complex, at the DME electrode with two protons involved (charges omitted for sake of simplicity) is:



The slope obtained between pH 8.2 and 8.8 (27 mV / pH) indicates that ZnL is formed. The reaction at the DME electrode with one proton involved results in a slope of 30 mV / pH:



In addition, the analysis of the variation of the shift in the corrected half-wave potential with the concentration of free ligand (insert in Figure 8-2B) confirms that AMPSO complexes zinc, as ZnL complex species, indicated by the slope of 31 mV / log[L]. From the analysis of Figure 8-2B, the species ZnL and ZnL_x(OH)₂ are expected to be formed. So, it is very likely that the ZnL_x(OH) species is also present in solution. The starting model should be ZnL, ZnL(OH) and ZnL(OH)₂.

The optimization of the final model and the refinement of the overall stability constants were done using the graphic analysis of the experimental (ECFC) and calculated (CCFC) complex formation curves (Figure 8-3B). The obtained results of the refined DCP data are summarized in Table 8-2. The

refinement of Model 1 (ZnL , ZnL(OH) and ZnL(OH)_2) resulted in the rejection of ZnL(OH) (Table 8-2). In Figure 8-3B, the ECF and CCF curves, for Model 1 for $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 609$, were drawn. The CCFC for $\text{Zn}_x(\text{OH})_y$ species was also included. The comparison between the ECFC and the CCFC for $\text{Zn}_x(\text{OH})_y$ species evidences unequivocally that AMPSO complexes zinc; the difference between the two curves at pH 8.0 is ≈ 4 mV and at pH 9.3 is of about 19 mV. Figure 8-3B also shows that CCFC for Model 1 (Table 8-2) fitted well the experimental data. Even though ZnL(OH) was rejected in Model 1, an attempt was made to include this species in the final model by fixing the value to the theoretical one (Model 2, Table 8-2). If one considers the hydrolyses of the complex ZnL , then $\log \beta_{\text{ZnL}} + \log \beta_{\text{Zn(OH)}} = \log \beta_{\text{ZnL(OH)}}$, this would come up with a value for $\log \beta_{\text{ZnL(OH)}}$ of 7.2. The inclusion of this species worsen the overall standard deviation of the fitting for both ratios (SD for Model 2, Table 8-2), which indicates that most likely this species is not formed in an appreciable extent in the experimental conditions described.

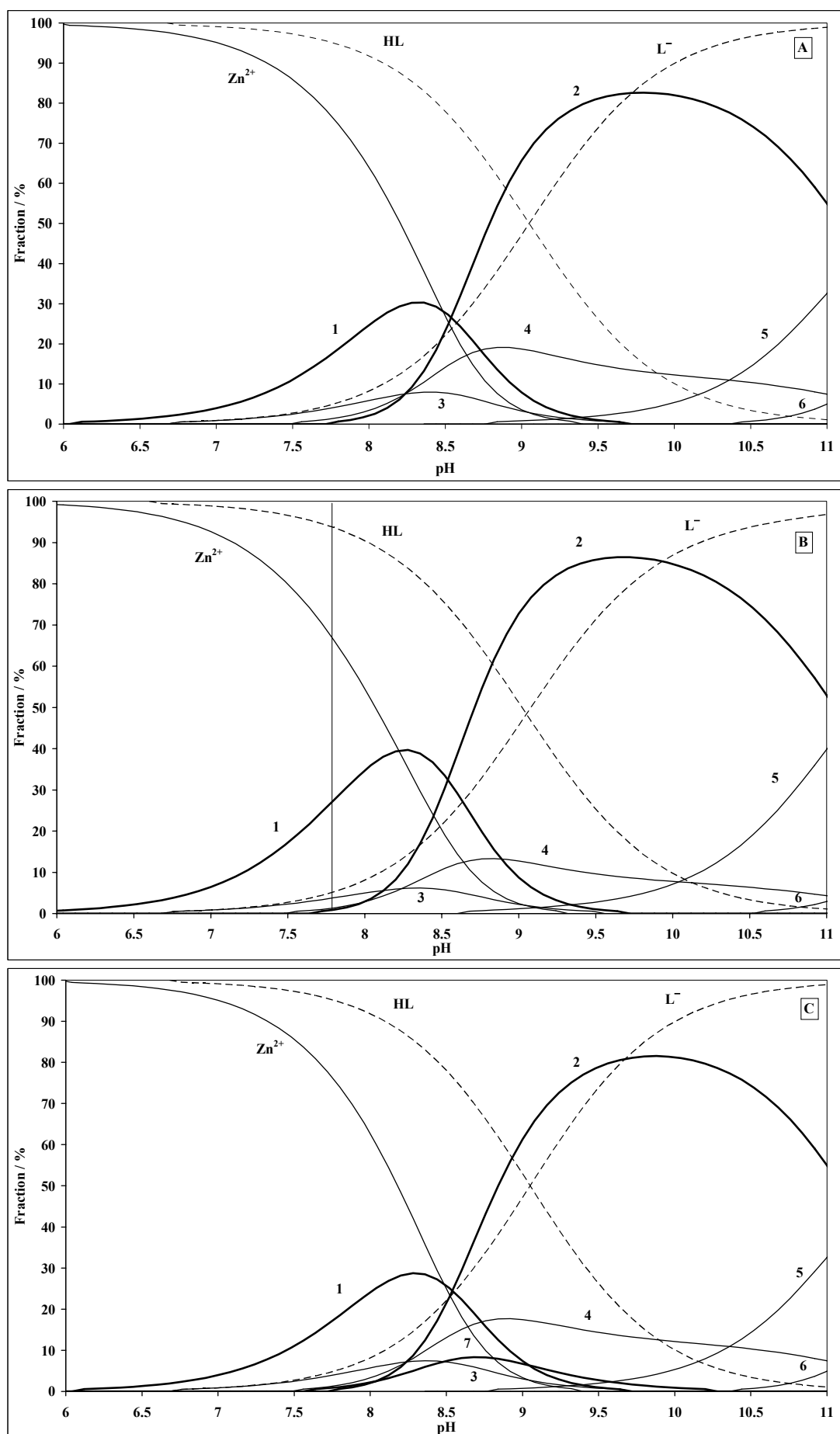
Table 8-2 Stability constants obtained for $\text{Zn}-(\text{AMPSO})_x-(\text{OH})_y$ system, determined at 25 °C and $I = 0.1$ M by DCP for $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 609$ and $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 799$, $[\text{Zn}^{2+}] = 2.5 \times 10^{-5}$ M (22 points, from pH 7.3 to 9.3 and 40 points, between pH 6.2 and 8.7, respectively) and GEP $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 25$, $[\text{Zn}^{2+}] = 1.0 \times 10^{-3}$ M (three titrations, 100 points, between pH 6.0 and 7.8).

Complexes / Equilibrium	DCP $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 609$		DCP $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 799$		GEP $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 25.0$
	Model 1	Model 2	Model 1	Model 2	
ZnL $\text{Zn} + \text{L} \rightleftharpoons \text{ZnL}$	2.49 ± 0.04	2.42 ± 0.05	2.56 ± 0.03	2.53 ± 0.04	$2.550 \pm 0.008^*$
ZnL(OH) $\text{Zn} + \text{L} + \text{OH} \rightleftharpoons \text{ZnL(OH)}$	R	7.2 (F)	R	7.2 (F)	NI
ZnL(OH)₂ $\text{Zn} + \text{L} + 2\text{OH} \rightleftharpoons \text{ZnL(OH)}_2$	12.78 ± 0.02	12.73 ± 0.03	13.02 ± 0.05	12.95 ± 0.06	NI
SD / mV	0.59	0.86	0.35	0.37	
Hamilton R-factor					0.0096

For DCP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (Part II, Chapter 2, Section 2.3.3). For GEP, Hamilton R-factor stands for a statistical Hamilton R-factor generated by the program ESTA (Part V, Chapter 10, Section 10.2.4.1). R- rejected; F – fixed value; NI – not included; * simultaneous refinement of acid concentration with a maximum variation of 0.4%

Using the stability constant values obtained from DCP experiments, a GEP experiment was designed. Even using an unusually high $[L_T]:[Zn_T]$ ratio, it was not possible to avoid precipitation, which occurred around pH 7.8; this fact made impractical the refinement of stability constants for other species than ZnL (Table 8-2). The stability constant value for ZnL refined by GEP is in perfect agreement with the refined values obtained by DCP (Table 8-2).

The final model considering results obtained by DCP and GEP experiments is: ZnL and ZnL(OH)₂, with stability constants set to (as log β values): 2.5 ± 0.1 and 12.9 ± 0.2 , respectively, where the standard deviations indicated refer to the interval of variation of the values of the stability constants. Using these values, species distribution diagrams for $[L_T]:[Zn_T]$ ratios 609 and 25 were plotted (Figure 8-5). Figure 8-5A evidences that, above pH 8.8, ZnL(OH)₂ is the main species, which is in agreement with the slope obtained in this range of pH (Figure 8-2). However, when we analyse the SDD in the pH range between 8.2 and 8.8, we verify that there is a mixture of several species. Thus, the slope of 27 mV / pH drawn in Figure 8-2B cannot be only attributed to ZnL, as it was anticipated, but a weighted average resulted from the several reduction reactions, which occur at the DME as a consequence of the mixture of species present in solution. For instance at pH 8.5, there is in solution: 26.9 % of free metal, 27.8 % of ZnL and 23.1 % of ZnL(OH)₂. As it was mentioned before, for GEP conditions, ZnL is the only ligand containing metal species present in solution, prior to precipitation, which was observed at about pH = 7.8 (Figure 8-5B). Additionally, SDD (Figure 8-5C) was generated for the final model (ZnL, ZnL(OH)₂) plus ZnL(OH) species with a stability constant set to the theoretical value. This SDD predicts, that if this complex were formed, only a small amount would be present in all the pH-range studied (the highest quantity predicted is 8.3% at pH 8.7).



In conclusion, the characterization of Cd-(AMPSO)_x-(OH)_y and Zn-(AMPSO)_x-(OH)_y systems proved to be a difficult task as the complexation for both systems occurs in the region of metal hydrolyses and in some cases M(OH)₂ precipitation. The fact that AMPSO protonation constant is very basic ($pK_a = 9.05$) and the complexes formed between cadmium or zinc and AMPSO are not too strong just added another challenge.

For both systems, the final models were only possible to be totally defined by DCP. Even using unusual high [L_T]:[M_T] ratios, GEP evidenced clear limitations and did not allow the refinement of all the stability constant values of the final models for Cd-(AMPSO)_x-(OH)_y and Zn-(AMPSO)_x-(OH)_y systems. The use of polarography allowed to go further and characterize properly both systems at higher pH values without occurrence of M(OH)₂ precipitation.

In addition, this study has shown that, in the pH range where AMPSO is used as a buffer (between pH 8.0 and 10.0), AMPSO can form several complexes with cadmium, CdL and CdL(OH), or zinc, ZnL and ZnL(OH)₂. These results should warn the users of this buffer, that AMPSO must not be used for studies involving cadmium or zinc ions in solution unless the extent of complexation is accounted for.

← **Figure 8-5** Species distribution diagrams computed for Zn-(AMPSO)_x-(OH)_y system. (A) and (C) DCP experimental conditions ([L_T]:[Zn_T] = 609 with [Zn²⁺] = 2.5 × 10⁻⁵ M); (B) GEP experimental conditions ([L_T]:[Zn_T] = 25.0 with [Zn²⁺] = 1.0 × 10⁻³ M). For (A) and (B), the metal-ligand model consisted of ZnL (1), ZnL(OH)₂ (2), Zn(OH) (3), Zn(OH)₂ (4), Zn(OH)₃ (5) and Zn(OH)₄ (6), for which stability constants, as log β, were set to 2.5, 12.9, 4.7, 10.2, 13.9 and 15.5, respectively. For (C), ZnL(OH) (7), with a stability constant value set to 7.2, was added to the metal-ligand model used for (A) and (B) speciation. The vertical line indicates the pH at which precipitation was observed.

9 INTERPRETATION OF NON-NERNSTIAN SLOPES IN GRAPHIC ANALYSIS OF DATA COLLECTED IN pH-RANGE OF DEPROTONATION OF A LIGAND. PART II. A GLASS ELECTRODE POTENTIOMETRIC AND POLAROGRAPHIC STUDY OF $\text{Cd}-(\text{TAPS})_x-(\text{OH})_y$ AND $\text{Zn}-(\text{TAPS})_x-(\text{OH})_y$ SYSTEMS⁸

ABSTRACT

In this work, the stability constants for cadmium or zinc and the biological buffer TAPS are reported for the first time. Both systems were studied by DCP and GEP, at fixed total ligand to total metal concentrations ratios and varied pH values, at 25.0 ± 0.1 °C and ionic strength 0.1M KNO_3 . Due to the weakness of the complexes formed and the high basicity of the ligand, full characterization of both $\text{M}-(\text{TAPS})_x-(\text{OH})_y$ systems, whose complexation occurs in the region of metal hydrolysis and $\text{M}(\text{OH})_2$ precipitation, was only possible to be performed by DCP using large $[\text{L}_\text{T}]:[\text{M}_\text{T}]$ ratios and low total metal concentrations. For $\text{Zn}-(\text{TAPS})_x-(\text{OH})_y$ system, two species, ZnL and $\text{ZnL}(\text{OH})_2$, which are major species in the buffering pH range, were identified. The overall stability constants values are 2.3 ± 0.1 and 13.0 ± 0.2 , respectively. For $\text{Cd}-(\text{TAPS})_x-(\text{OH})_y$ system, CdL , with $\log \beta = 2.40 \pm 0.04$, was the only species detected up to pH 9.5. A comparative analysis of the stability constants between several biological buffers was done.

9.1 INTRODUCTION

The compound TAPS belong to a long list of biological buffers widely used in biochemistry, biological and chemistry works. All these buffers are β aminoalcohols, which make them as potential metal complexing agents. Nowadays, some of these buffers are presently used routinely in biochemical and speciation studies under the assumption that they undergo little, or if any, interaction with biologically/environmentally important ions. However, several reports have described interferences when some of these buffers were used in the presence of metal ions.

The knowledge of the stability of metal-(pH buffer) complexes is of fundamental significance when modelling of natural waters, biological fluids, industrial processes or ligand design strategies are of interest.

Since 1997, we started a project, whose central aim is to study the interaction of these buffers with

⁸ Submitted for publication

metal ions and their suitability for metal speciation and biological/biochemical studies involving metal ions. Up till now, several pairs of metal ions–buffers have already been studied and important conclusions were drawn. Among them, TAPSO and TAPS, which are structural related (TAPSO has only one more hydroxyl in the second carbon), revealed to be strong copper (Part II, Chapter 2) and lead (Part III, Chapter 5) complexing agents in the buffering pH range. An appreciable complexation between cadmium or zinc and TAPSO was also reported (Part III, Chapter 7).

In the present work, the overall stability constants for the species formed between TAPS and Cd^{2+} or Zn^{2+} ions, at 0.1 M KNO_3 ionic strength and $25.0 \pm 0.1^\circ\text{C}$, are reported for the first time. The characterization of the systems was done by DCP and GEP. Experiments by ACP were also carried out to check if this buffer adsorbs at the mercury working electrode surface, under the experimental conditions used.

9.2 EXPERIMENTAL

9.2.1 MATERIALS AND REAGENTS

The standard metals solutions, cadmium (1.8×10^{-2} M) and zinc (1.5×10^{-2} M), were purchased from Merck.

Additional information about the materials and reagents were reported previously (Part II: Chapter 2, Section 2.2.1, and Chapter 4, Section 4.2.1).

9.2.2 APPARATUS

All experiments were carried out in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer, and thermostatted at $25.0 \pm 0.1^\circ\text{C}$ using a water bath.

9.2.2.1 POLAROGRAPHY

Polarographic measurements, as well as alternating current studies, were performed as previously described (Part II, Chapter 4, Section 4.2.2.1).

9.2.2.2 POTENTIOMETRY

The potentiometric titrations were performed as described previously (Part II, Chapter 2, Section 2.2.2.2).

9.2.3 PROCEDURE

9.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

For all techniques used, the calibration of the glass electrode (pH measured as $-\log [H^+]$) was accomplished as previously described (Part II, Chapter 3, Section 3.2.3.1).

9.2.3.2 ALTERNATING CURRENT POLAROGRAPHY

In order to evaluate the adsorption of the ligand TAPS on the surface of the mercury of the working electrode, ACP polarograms were run in the absence of the metal ion. The experimental conditions used were described elsewhere (Part II, Chapter 4, Section 4.2.3.2).

9.2.3.3 DIRECT CURRENT POLAROGRAPHY

The study of the electrochemical behaviour of Cd-(TAPS)_x-(OH)_y and Zn-(TAPS)_x-(OH)_y systems was performed by DCP. The procedure used to run the polarographic titrations was described previously (Part II, Chapter 2, Section 2.2.3.3). For Cd-(TAPS)_x-(OH)_y system, a $[L_T]:[Cd_T] = 200.0$, with $[Cd^{2+}] = 1.0 \times 10^{-5}$ M, and $[L_T]:[Cd_T] = 375.6$, with $[Cd^{2+}] = 4.0 \times 10^{-5}$ M, were used. The pH was varied between 5.5 and 9.5 and about 30 points were collected, for each titration. The electrochemical behaviour of the Zn-(TAPS)_x-(OH)_y system was analysed using two different $[L_T]:[Zn_T]$ ratios: 794.2 and 675.5, $[Zn^{2+}] = 2.5 \times 10^{-5}$ M and 2.0×10^{-5} M, respectively. The pH was varied from 6.0 to 9.1; for each titration, about 35 points were collected. A step potential of 4 mV were used for all metal-TAPS systems. A drop time of 1s was used for all the systems except for the highest ratio used for Cd-(TAPS)_x-(OH)_y system, where it was used a drop time of 2s. The equilibrium of the metal-ligand solutions was tested and it was reached in a few minutes.

9.2.3.4 GLASS ELECTRODE POTENTIOMETRY

For both $M^{2+}-(TAPS)_x-(OH)_y$ systems studied, several potentiometric titrations were performed using monotonic titrant additions of standardized KOH 0.1M. For Cd–(TAPS)_x–(OH)_y system, a $[L_T]:[Cd_T] = 6.0$, $[Cd^{2+}] = 1.0 \times 10^{-3}$ M, was used. Four titrations, using two independent solutions, were performed; for each titration, about 70 points were collected between pH 5.0 and 8.5. For Zn–(TAPS)_x–(OH)_y system, two independent solutions with $[L_T]:[Zn_T] = 15.5$, $[Zn^{2+}] = 9.9 \times 10^{-4}$ M were used. For each titration, about 80 points were collected between pH 6.0 and 8.0; a total of five titrations were performed.

9.2.4 DATA TREATMENT

The refinement of polarographic data was performed using the Cukrowski method [9, 10]; as it was described previously (Part II, Chapter 2, Section 2.2.4).

The simulation and optimization procedures of potentiometric data was done using ESTA program [7, 8]; for more details about the program see Part II, Chapter 2, Section 2.3.3 and Part V, Chapter 10, Section 10.2.4.1.

During the refinement of the overall stability constants for both systems, the water [11] (Part II, Chapter 2, Table 2-1) and ligand (Part II, Chapter 4, Table 4-1) protonation constants, as well as all known stability constants for $Cd_x(OH)_y$ and $Zn_x(OH)_y$ species [11] (Table 7-1), were kept fixed.

9.3 RESULTS AND DISCUSSION

9.3.1 LIGAND ADSORPTION AND METAL REVERSIBILITY STUDIES

Because stability constants obtained by polarographic techniques can be significantly influenced by the ligand adsorption, the possible adsorption of TAPS at the mercury electrode was studied by ACP at a phase angle of 90°. The adsorption effect was quantified by comparing the AC polarograms for the background solution with the polarograms obtained with increasing ligand additions. Figure 9-1 shows the variation in capacitive current, expressed in percentage, obtained with the increase of ligand concentration relatively to the background solution (0.1 M KNO₃), for reduction potentials typical of Cd²⁺ and Zn²⁺ ions (–0.54 and –0.96V, respectively [18]), at pH 6.34 (Figure 9-1A), pH 8.34 (Figure 9-1B) and pH 10.34 (Figure 9-1C). A global analysis of Figure 9-1 shows that the highest variation in the capacitive current with the increase of the ligand concentration was recorded at pH =

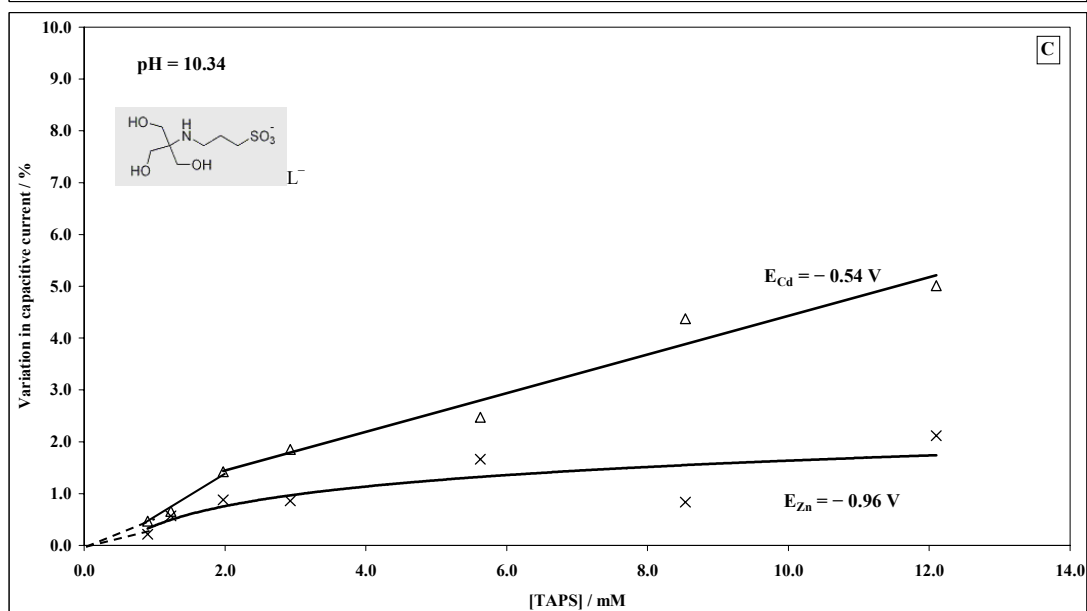
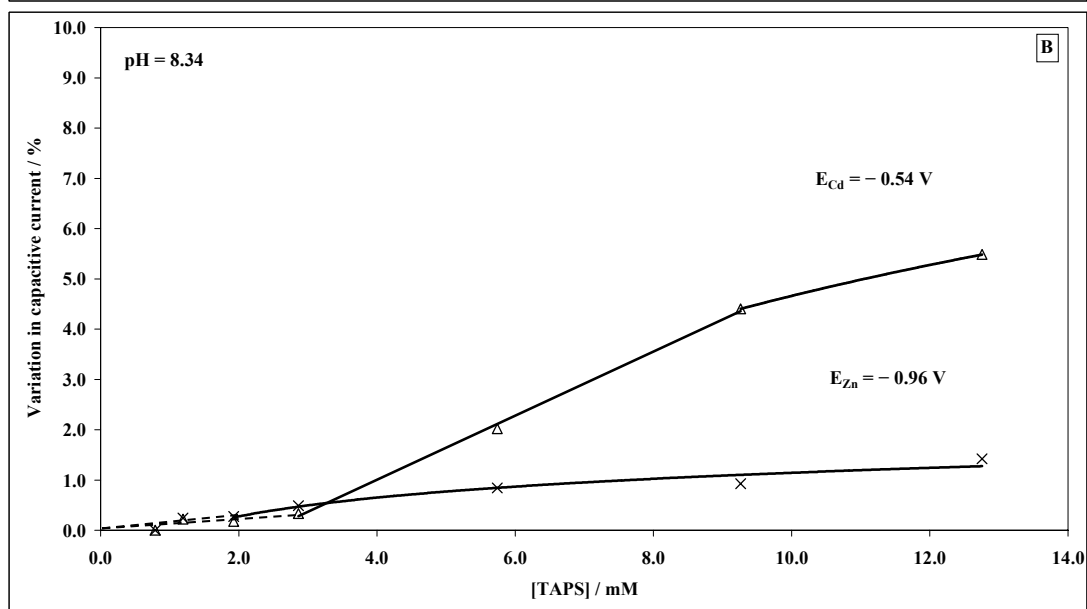
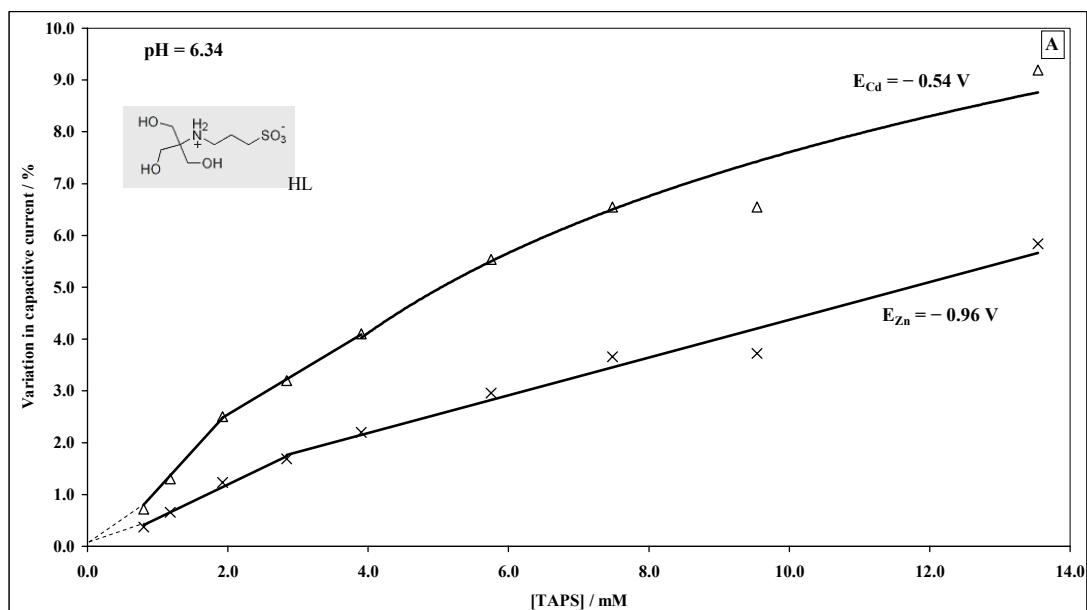
6.34 for both potential reductions, being this effect slightly more marked for cadmium than zinc potential reductions (less than 10% for both metal ions in all the TAPS concentration range tested). For potential reduction typical of zinc, no significative adsorption was detected at pH 8.34 and 10.34 (a variation in capacitive current less than 2% was recorded). The reduction of cadmium and zinc ions occurs at potential values more cathodic than the zero charge potential (-0.4V), where the surface of the mercury electrode is negatively charged [19], which means that adsorption of cations is favoured. For pH = 6.34 (Figure 9-1A), the ligand is totally in the protonated form, HL (insert in Figure 9-1A), whereas for pH 10.34, it is 100% in the deprotonated form, which means that it has negative charge, L^- (insert in Figure 9-1C). The fact that an increase of the adsorption effect was observed with the decrease of pH, suggests that at these potential values, -0.54 and -0.96V , the adsorption of TAPS is mainly due to the positive charged part of the ligand, the amine group (insert in Figure 9-1A). The results described above pointed out that up to a TAPS concentration of $1.4 \times 10^{-2}\text{ M}$, no significant adsorption of TAPS at the potential region where cadmium and zinc are reduced, was observed.

For $\text{Cd}-(\text{TAPS})_x-(\text{OH})_y$ system, fully reversible DC polarograms were recorded between pH 5.5 and 9.5; wave steepness values varied between 0.95 and 1.0, throughout the pH.

For $\text{Zn}-(\text{TAPS})_x-(\text{OH})_y$ system, a decrease in the wave steepness values from 0.95 to 0.71 recorded between pH 6.0 and 9.1, respectively, evidenced a quasi-reversibility of the metal reduction at the mercury electrode surface. In order to use the data of this system and since the limiting diffusion current does not depend on the degree of reversibility, a small correction of the irreversibility, using a procedure previously described (Part II, Chapter 3, Section 3.3.1) was done.

9.3.2 MODELLING AND OPTIMIZATION OF THE STABILITY CONSTANTS FOR $\text{M}-(\text{TAPS})_x-(\text{OH})_y$ SYSTEMS

For DCP data and for both systems studied, the data analysis collected in all the pH range tested showed that $\text{M}-(\text{TAPS})_x-(\text{OH})_y$ systems behaved as labile and dynamic on the polarographic time scale; only one peak corresponding to all labile species present in solution, which shifted to more cathodic potential values, was observed (data not shown).



9.3.2.1 Cd-(TAPS)_x-(OH)_y SYSTEM

For Cd-(TAPS)_x-(OH)_y system, two DCP titrations were performed: [L_T]:[Cd_T] = 200.0, with [Cd²⁺] = 1.0 × 10⁻⁵ M and [L_T]:[Cd_T] = 375.6, with [Cd²⁺] = 4.0 × 10⁻⁵ M. The following analysis is concerned to the lowest [L_T]:[Cd_T] ratio; similar results were obtained for the other [L_T]:[Cd_T] ratio (data not shown).

In Figure 9-2, the analysis of the variation of the observed half-wave potential shift with the increase of the pH is represented. Ligand protonation constant and the different forms of the ligand according to the pH values were also marked in the figure. The possible reactions at the dropping mercury electrode (DME), according to the pH of the solution, are also marked in the figure (charges were omitted for sake of simplicity). The analysis of data points showed that TAPS does not bind strongly with cadmium; the total shift in the half-wave potential, up to pH 9.5, was only 7.6 mV (Figure 9-2A). Only one slope could be detected in all the pH range tested (Figure 9-2A); between pH 7.2 and 9.1, a slope of about 3 mV per pH unit was drawn, indicating some interaction between cadmium and TAPS. The expected species to be formed in this pH range are CdL and Cd(OH). Theoretical slope for CdL would be 29.5 mV/pH (Figure 9-2A), since only one proton is involved in the electrochemical reaction. The explanation for this low slope lies in the fact that in this pH range, the deprotonation of the ligand has already started, and up to pH 9.1 around 80% of TAPS is deprotonated. So, the reaction with no proton involvement should also be considered, where no slope is developed (Figure 9-2A). One must also consider the formation of Cd(OH) species, which is expected to be formed above pH 8.0 (see Figure 9-3A). The presence of this species in solution explains the observed slope up to pH 9.5. If only CdL species was present in solution and considering the slope explanation previously presented, a decrease of slope to zero would be observed, and this was not the case (Figure 9-2A).

← **Figure 9-1** Variation in the capacitive current recorded by ACP, without the metal ion, with the increase of TAPS concentration at different pH values: 6.34 (A), 8.34 (B) and 10.34 (C). The values, expressed in percentage, were calculated from the difference between the capacitive current recorded in the absence and in the presence of the buffer, at potential values indicated in the figure, from ACP curves with a phase angle of 90° in 0.1 M KNO₃.

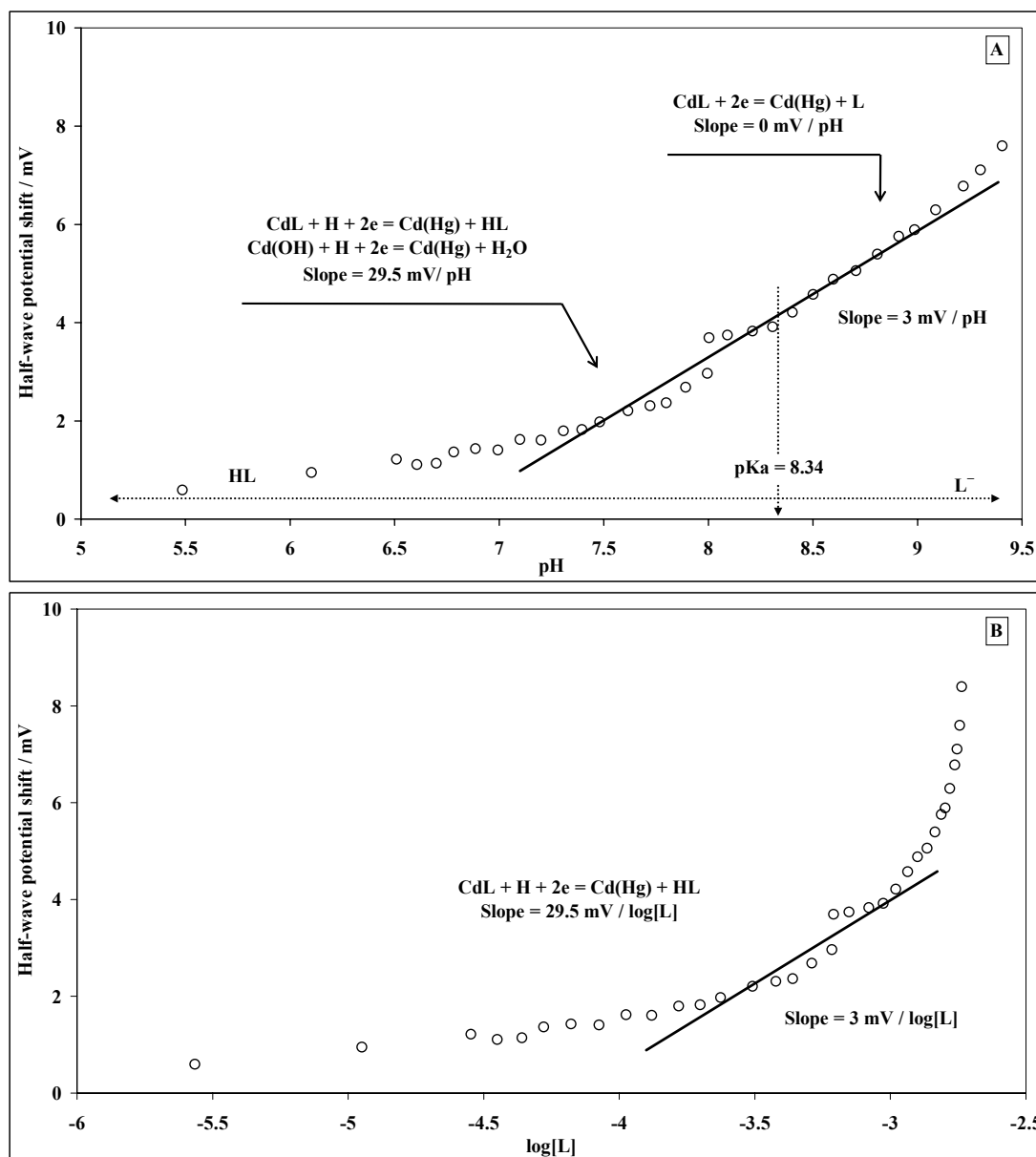


Figure 9-2 Analysis of DCP data obtained from the $\text{Cd}-(\text{TAPS})_x-(\text{OH})_y$ system for $[\text{L}_\text{T}]:[\text{Cd}_\text{T}] = 200.0$, $[\text{Cd}^{2+}] = 1.0 \times 10^{-5} \text{ M}$, at 25.0°C and 0.1 M KNO_3 ionic strength. Half-wave potential shift as a function of (A) pH and (B) computed free ligand concentration.

The analysis of the observed half-wave potential shift vs. $\log[\text{L}]$ (Figure 9-2B), where a slope of $3.0 \text{ mV}/\log[\text{L}]$ was drawn, corroborates that CdL seems to be formed but not as a major species. For this system, graphical analysis, performed between pH 7.2 and pH 9.5, only pointed out the formation of CdL .

The obtained results for DCP data, for both DCP titrations, are summarized in Table 9-1. Because in similar systems, $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ (Part III, Chapter 7), $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ (Part III, Chapter 8) and $\text{Cd}-(\text{Triethanolamine})_x-(\text{OH})_y$ [11], the presence of CdL_2 and/or CdL(OH) complexes

was verified, we decided to include these species in the refinement operations. All attempts resulted in the rejection of both species. In order to confirm the stability constant value for CdL refined by DCP data, GEP experiments were also carried out. Since this technique works at much lower [L_T]:[Cd_T] ratios, information regarding the possible formation of CdL(OH) species could be obtained by this technique. Even in these conditions, the presence of other species besides CdL was not detected. The refined stability constant for CdL value was in perfect agreement with the DCP data (Table 9-1).

Table 9-1 Refinement of the stability constants (as log β) for Cd-(TAPS)_x-(OH)_y system by DCP ([L_T]:[Cd_T] = 200.0, [Cd²⁺] = 1.0 × 10⁻⁵ M, 30 points, from pH 6.5 to 9.5 and [L_T]:[Cd_T] = 375.6, [Cd²⁺] = 4.0 × 10⁻⁵ M, 45 points, from pH 7.0 to 9.3) and GEP ([L_T]:[Cd_T] = 6.0, [Cd²⁺] = 1.0 × 10⁻³ M, four titrations, 238 points, from pH 6.0 to 8.5) titrations.

Complexes / Equilibrium	DCP		GEP
	[L _T]:[Cd _T] = 200.0	[L _T]:[Cd _T] = 375.6	[L _T]:[Cd _T] = 6.0
CdL Cd + L ⇌ CdL	2.40 ± 0.04	2.40 ± 0.01	2.433 ± 0.007
CdL₂ Cd + 2L ⇌ CdL₂	R	R	NI
CdL(OH) Cd + L + OH ⇌ CdL(OH)	R	R	NI
SD / mV	0.50	0.61	
Hamilton R-factor			0.0077

For DCP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (Part II, Chapter 2, Section 2.3.3). For GEP, Hamilton R-factor stands for a statistical Hamilton R-factor generated by the program ESTA (Part V, Chapter 10, Section 10.2.4.1). R – rejected; NI – not included

Considering the average of the refined values obtained by both techniques, the final model and overall stability constant value, for Cd-(TAPSO)_x-(OH)_y system is: log $\beta_{CdL} = 2.40 \pm 0.04$.

To test the proposed model, SDD were generated for [L_T]:[Cd_T] conditions used in DCP ([L_T]:[Cd_T] = 200.0) and GEP experiments assuming the final model proposed (log $\beta_{CdL} = 2.40$) (Figure 9-3).

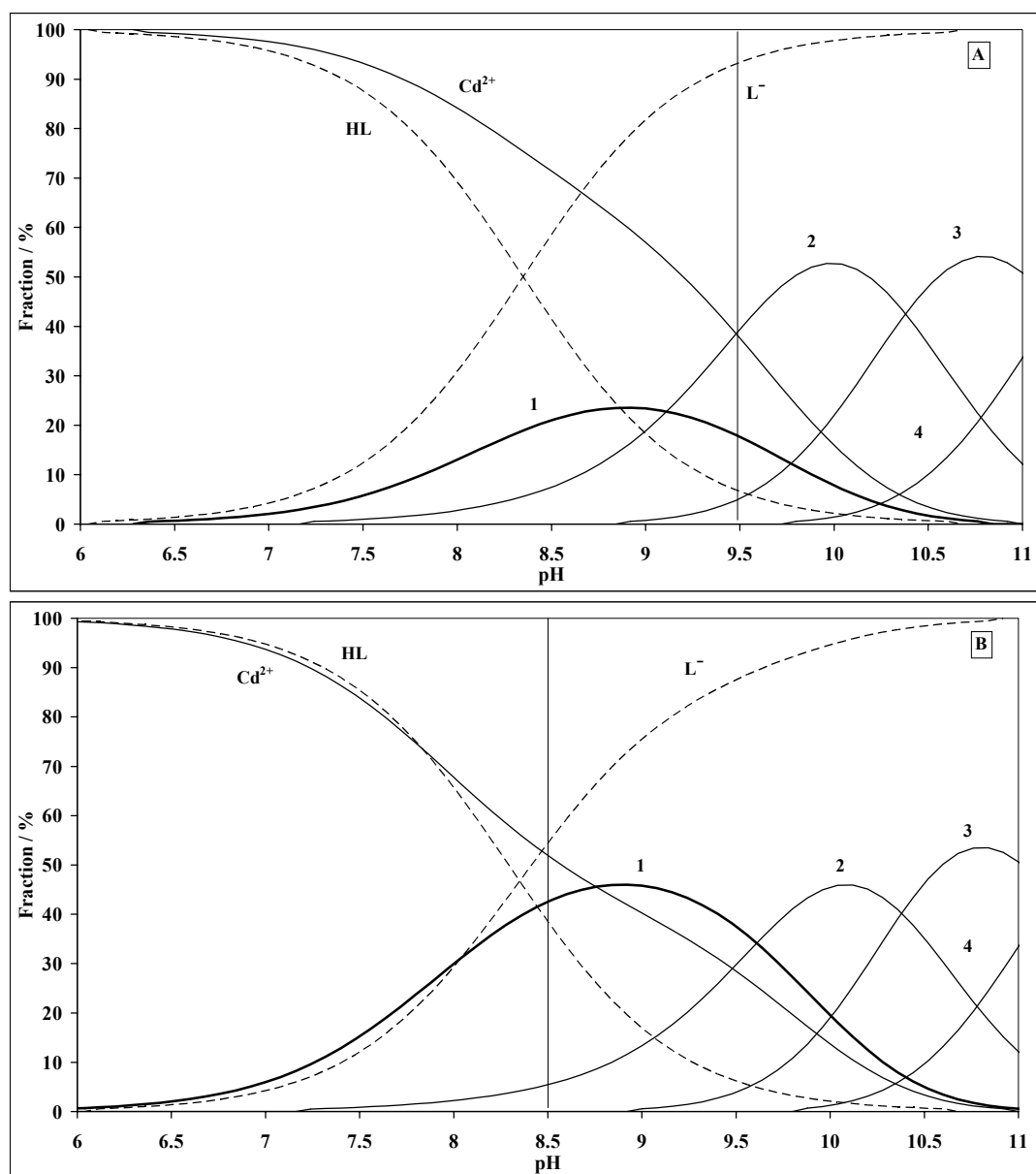


Figure 9-3 Species distribution diagrams for the final model for Cd-(TAPS)_x-(OH)_y system. A. $[L_T]:[Cd_T] = 200.0$, $[Cd^{2+}] = 1.0 \times 10^{-5}$ M. B. $[L_T]:[Cd_T] = 6.0$, $[Cd^{2+}] = 1.0 \times 10^{-3}$ M. Stability constants set to (as $\log \beta$): $CdL = 2.40$ (1), $Cd(OH)$ (2), $Cd(OH)_2$ (3), $Cd(OH)_3$ (4) ($\log \beta_{Cd(OH)_y}$ included in Table 7-1).

For $[L_T]:[Cd_T] = 200.0$, SDD (Figure 9-3A) predicts that, complexation starts at about pH 7.5, being CdL and $Cd(OH)$ the only species present in solution prior the precipitation to occur at about pH 9.5, and this was experimentally observed. This figure also shows that the exponential increase of CdL only occurs just after the deprotonation of the ligand in straight competition with metal ion hydrolysis. This fact explains why no other species could be formed. The SDD for GEP conditions (Figure 9-3B) shows the predominance of CdL species in the pH region analyzed.

9.3.2.2 Zn-(TAPS)_x-(OH)_y SYSTEM

For this system, two polarographic titrations were done, $[L_T]:[Zn_T] = 794.2$ and 675.5 , with $[Zn^{2+}] = 2.5 \times 10^{-5}$ and 2.0×10^{-5} M, respectively, in the pH range between 6.0 and 9.1.

After the correction of the waves regarding reversibility (for more details see Part II, Chapter 3, Section 3.3.1), the modelling of Zn-(TAPS)_x-(OH)_y system started with the graphical analysis of the experimental data for both $[L_T]:[Zn_T]$ ratios. For $[L_T]:[Zn_T] = 675.5$, the variation of the corrected half-wave potential shift as a function of pH and $\log [L]$ is illustrated in Figure 9-4. Similar results were obtained for the other ratio (data not shown). In Figure 9-4, TAPS protonation constant, as well as the different forms of the ligand according to the pH of the solution, were also included. In addition, all reasonable reactions at the DME electrode, which could explain the experimental slopes, were also included in Figure 9-4 (for sake of simplicity charges were omitted). From the analysis of Figure 9-4A, two slopes were identified. The first slope, 15.5 mV/pH, was drawn between pH 7.4 and pH 8.4. This slope must be due to the presence of ZnL since the reaction at the DME electrode would involve one proton developing a theoretical slope of 29.5 mV / pH (Figure 9-4A). Because the pK_a of the ligand is 8.34, in this pH region, part of the ligand is already deprotonated and the reduction of ZnL, in this case, would generate no slope, since no protons are involved in the DME reaction, $ZnL + 2e = Zn(Hg) + L$. This is the reason why the slope did not develop to its theoretical value. One must also consider the formation of Zn(OH) species, that is expected to be formed above pH 7.5 (Figure 9-5A) and for which reaction at the DME electrode would also involve one proton. In the pH range between 8.4 and 9.1, a slope of 42.3 mV / pH was drawn. In this pH range, TAPS is mainly in the deprotonated form (Figure 9-4A). In this pH range, a mix of several species ($ZnL_x(OH)$, $ZnL_x(OH)_2$, $Zn(OH)$ and $Zn(OH)_2$) can be present in solution. The generated experimental slope is too high to be only explained by the reduction of $ZnL_x(OH)$ and too low to be just due to $ZnL_x(OH)_2$ and/or $Zn(OH)$ and $Zn(OH)_2$ (Figure 9-4A). In principle, reduction of $Zn(OH)_2$ should be excluded as a major species since the polarographic signal shifted up to pH 9.1 and no precipitation was observed. The simpler and most likely explanation for this slope is the simultaneous existence in solution of $ZnL_x(OH)$ and $ZnL_x(OH)_2$ species. The analysis of the corrected half-wave potential shift with the variation of $\log [L]$ (Figure 9-4B) shows a slope of 21.6 mV / $\log [L]$, between $\log [L] -2.8$ and -2.2 , which corresponds to the pH range between 7.4 and 8.3, corroborating the presence of ZnL species (Figure 9-4A) in solution.

From the analysis of data described in Figure 9-4, the most likely species present in solution are: ZnL, ZnL(OH) and ZnL(OH)₂.

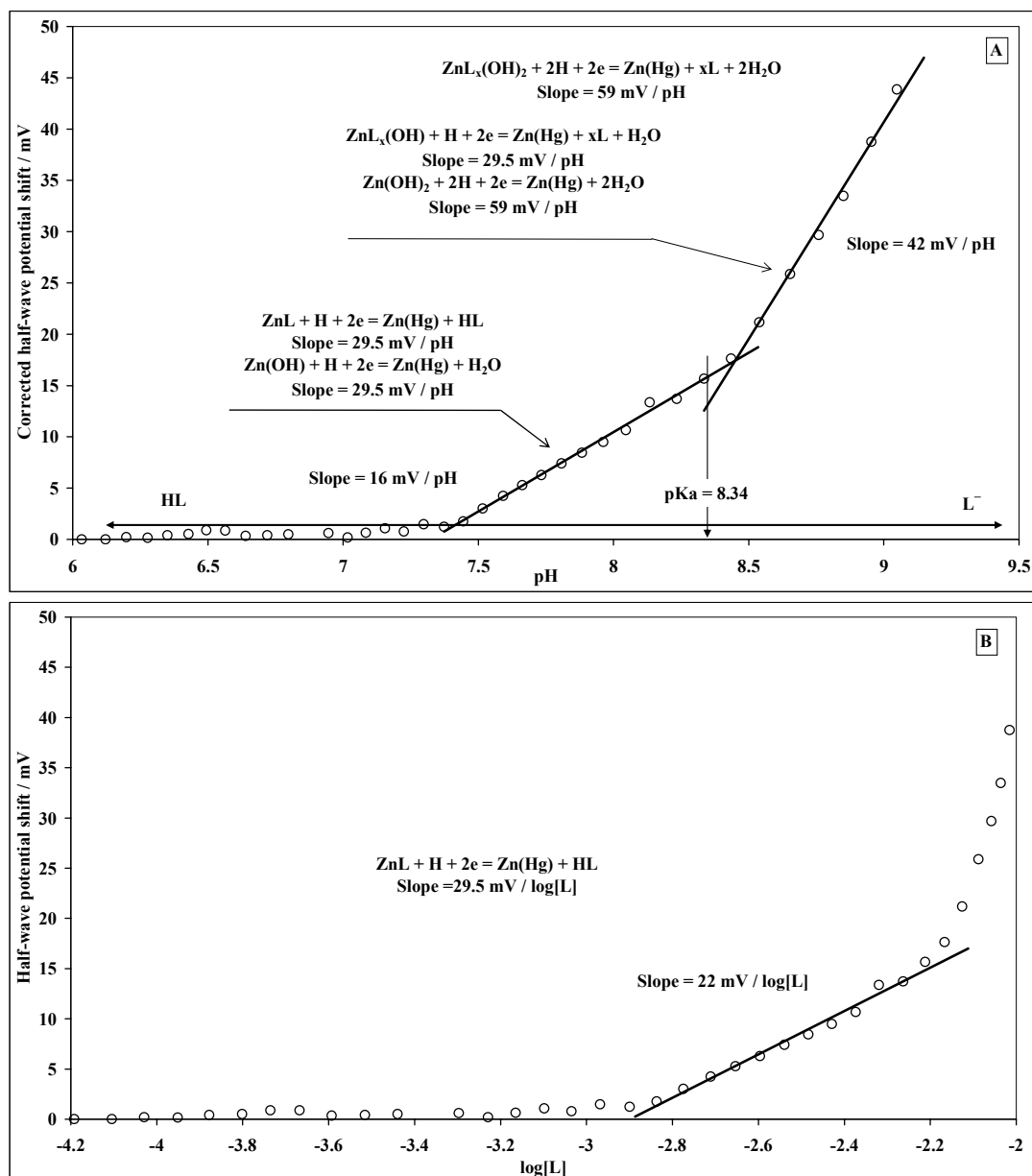


Figure 9-4 Analysis of DCP data obtained from the $\text{Zn}-(\text{TAPS})_x-(\text{OH})_y$ system for $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 675.5$, $[\text{Zn}^{2+}] = 2.0 \times 10^{-5} \text{ M}$, at 25.0°C and 0.1 M KNO_3 ionic strength. Corrected half-wave potential shift as a function of (A) pH and (B) computed free ligand concentration

Summarized results for both polarographic titrations are compiled in Table 9-2. For both ratios, $\text{ZnL}(\text{OH})$ species was rejected, which indicates that in the pH range analyzed, it does not exist in sufficient amount in solution hindering the refinement of its stability constant. Considering that the formation of $\text{ZnL}(\text{OH})$ results from the hydrolyses of ZnL , then the stability constant for this species can be calculated by: $\log \beta_{\text{ZnL}(\text{OH})} = \log \beta_{\text{ZnL}} + \log \beta_{\text{Zn}(\text{OH})}$. The inclusion of $\log \beta_{\text{ZnL}(\text{OH})}$, as a fixed value (Table 9-2, Model 2), confirmed that no appreciable amount of this species is formed in solution, since no significant change in the other stability constant values and in the overall standard deviation were observed. Therefore, the model that better explains the DCP results is: $\text{ZnL} + \text{ZnL}(\text{OH})_2$.

Table 9-2 Refinement of the stability constants (as log β) for Zn-(TAPS)_x-(OH)_y system by DCP ($[L_T]:[Zn_T] = 794.2$, $[Zn^{2+}] = 2.5 \times 10^{-5}$ M, 33 points, from pH 6.2 until pH 9.1; $[L_T]:[Zn_T] = 675.5$, $[Zn^{2+}] = 2.0 \times 10^{-5}$ M, 37 points, from pH 6.0 until pH 9.1) and GEP ($[L_T]:[Zn_T] = 15.5$, $[Zn^{2+}] = 9.9 \times 10^{-4}$ M, 3 titrations, 151 points, from pH 6.0 until 7.3) titrations.

Complexes / Equilibrium	DCP				GEP
	$[L_T]:[Zn_T] = 794.2$		$[L_T]:[Zn_T] = 675.5$		$[L_T]:[Zn_T] = 15.5$
	Model 1	Model 2	Model 1	Model 2	
ZnL $Zn + L \rightleftharpoons ZnL$	2.24 ± 0.02	2.20 ± 0.02	2.41 ± 0.02	2.38 ± 0.03	$2.251 \pm 0.009^*$
ZnL(OH) $Zn + L + OH \rightleftharpoons ZnL(OH)$	R	6.9 (F)	R	7.1 (F)	NI
ZnL(OH)₂ $Zn + L + 2OH \rightleftharpoons ZnL(OH)_2$	12.83 ± 0.02	12.79 ± 0.02	13.11 ± 0.02	13.07 ± 0.02	NI
SD / mV	1.49	1.57	1.21	1.02	
Hamilton R-factor					0.00102

For DCP, SD represents an overall fit of complex formation curves, the computed curve in the objective experimental function (Part II, Chapter 2, Section 2.3.3). For GEP, Hamilton R-factor stands for a statistical parameter generated by the program ESTA (Part V, Chapter 10, Section 10.2.4.1). R – rejected; F – fixed value; * - with refinement of proton concentration, variation less than 1.5%; NI – not included

In order to confirm the stability constants values obtained for Zn-(TAPS)_x-(OH)_y system by DCP, predictions based in SDD, using the model refined by polarography (Model 1, Table 9-2), were made for designing the best analytical conditions for GEP experiments. The SDD (data not shown) predicted precipitation of Zn(OH)₂ at about pH 8.0 and indicated that only ZnL stability constant would be possible to establish prior to precipitation. Because complexation starts in the deprotonation range of the ligand and in order to avoid that the titration goes towards the deprotonation of the ligand and not to neutralise protons liberated from the zinc complexation (for example: $Zn + HL = ZnL + H$), data points up to the pH where the expected $[L]/\left[\sum_{x,y=0}^2 ZnL_x(OH)_y\right]$ ratio was lower than 6 were used. GEP titrations using a $[L_T]:[Zn_T] = 15.5$ ($[Zn^{2+}] = 9.9 \times 10^{-4}$ M) were run between pH 6.0 and 8.0. The refinement results of three titrations, using two independent solutions, were included in Table 9-2. To assure that $[L]/\left[\sum_{x,y=0}^2 ZnL_x(OH)_y\right]$ ratio was lower than 6, only data points collected between pH 6.0 and 7.3 were refined. GEP only allowed confirming the presence of ZnL in solution with a stability constant value comparable with the log β_{ZnL} values obtained by polarography (Table 9-2).

The combination of all the results refined from both techniques indicates that the final model for Zn-(TAPS)_x-(OH)_y system is: ZnL and ZnL(OH)₂. The values for the overall stability constants with the standard deviations calculated taking into account the various experiments performed are: log β_{ZnL}

$$= 2.3 \pm 0.1, \log \beta_{\text{ZnL}(\text{OH})_2} = 13.0 \pm 0.2.$$

Using the final model, SDD for $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 675.5$ and 15.5 were simulated and plotted in Figure 9-5. For DCP, Figure 9-5A evidences that, up to pH 8.4, ZnL is the main species present in solution. The fact that, at this pH, 50% of the ligand is in the deprotonated form, explains well the slope of 15.5 mV/pH (Figure 9-4A). Between pH 8.4 and 9.1, there is an exponential increase of $\text{ZnL}(\text{OH})_2$. The coexistence of a small amount of ZnL still present in solution with $\text{ZnL}(\text{OH})_2$ explains why the theoretical slope typical of $\text{ZnL}(\text{OH})_2$ reduction, 59 mV / pH , was not developed (Figure 9-4A). Figure 9-5B clearly evidences that no other species, besides ZnL, was possible to be refined under GEP experimental conditions. These SDD point out that, in the pH range between 7.7 and 9.1, which corresponds to the TAPS' buffering pH region, ZnL and $\text{ZnL}(\text{OH})_2$, are major species in solution. Thus, the stability constants values of these species should be account for in speciation studies where zinc is involved and TAPS is used as a buffer.

In conclusion, the polarographic technique of speciation and refinement of stability constants employed in this work proved to be more powerful than the well-established procedures employed in GEP. After a simple correction performed on the experimental data, already employed successfully in a previous work [9], the use of DCP became determinant for full and adequate characterization of the systems due to the following reasons (i) complexation occurs in the region of metal hydrolysis and $\text{M}(\text{OH})_2$ precipitation; (ii) ligands are very simple with no double bonds, which disabled the use of fluorescence and UV-Vis for which low concentration of ligands (between 10^{-7} and 10^{-4} M) could be applied if ligands absorbed; (iv) weak complexes were expected to be formed and (v) the combination of potentiometry and polarography allowed us to cover a wide range of total metal concentration (between 10^{-5} and 10^{-3} M) and a wide range of $[\text{L}_\text{T}]:[\text{M}_\text{T}]$ ratios.

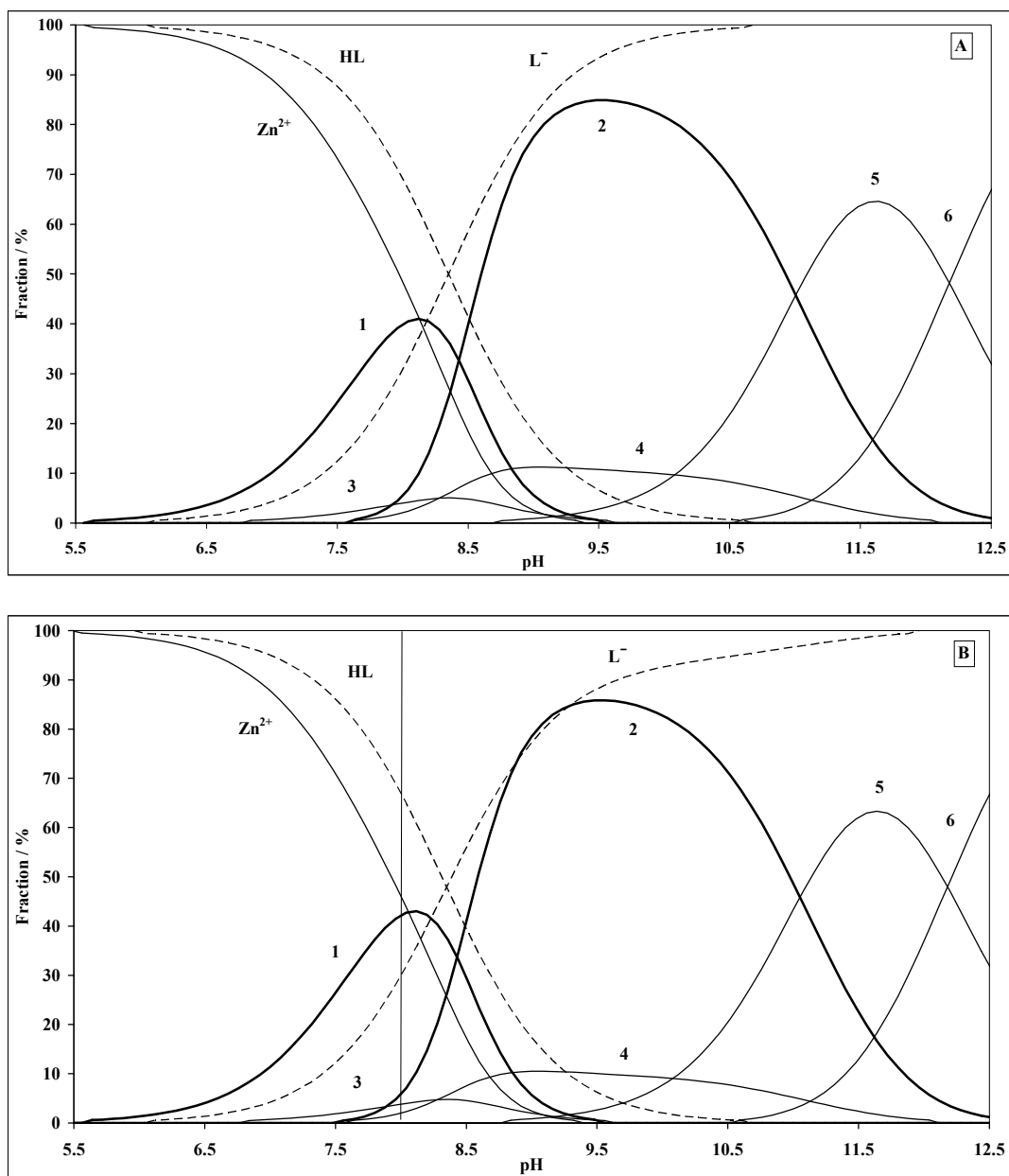


Figure 9-5 Species distribution diagrams for the final model for $\text{Zn}-(\text{TAPS})_x-(\text{OH})_y$ system. A. $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 675.5$, $[\text{Zn}^{2+}] = 2.0 \times 10^{-5}$ M. B. $[\text{L}_\text{T}]:[\text{Zn}_\text{T}] = 15.5$, $[\text{Zn}^{2+}] = 9.9 \times 10^{-4}$ M. Stability constants set to (as $\log \beta$): $\text{ZnL} = 2.3$ (1), $\text{ZnL}(\text{OH})_2 = 13.0$ (2), $\text{Zn}(\text{OH})$ (3), $\text{Zn}(\text{OH})_2$ (4), $\text{Zn}(\text{OH})_3$ (5), $\text{Zn}(\text{OH})_4$ ($\log \beta_{\text{Zn}(\text{OH})_y}$ included in Table 7-1).

9.3.3 CORRELATION BETWEEN THE LIGAND BASICITY AND COMPLEX STABILITY OF SEVERAL CdL AND ZnL SYSTEMS

Complex stability constants values for CdL and ZnL and protonation constants for some biological buffers, all structurally similar to TAPS, AMPSO, TRIS, TAPSO, TES, BIS-TRIS and other amines are compiled in Table 9-3.

Table 9-3 Comparison between CdL and ZnL complex stability constants and basicity for some biological buffers and other amines.

Ligand	Structures	pKa	Log β_{CdL}	Log β_{ZnL}	References
Methylamine	H_2NCH_3	10.65	2.75	*	[11]
Ethanolamine	$\text{H}_2\text{NCH}_2\text{CH}_2\text{OH}$	9.52	2.65	2.41	[11]
Ammonia	NH_3	9.28	2.56	2.31	[11]
AMPSO		9.05	2.1	2.5	Chapter 8
TAPS		8.34	2.4	2.3	This work
THAM or TRIS		8.10	1.88	2.27	[11]
TAPSO		7.55	2.2	2.5	Chapter 7
TES		7.5	**	2.08	[11]
BIS-TRIS		6.54	2.47	2.38	[11]

* Published data do not meet criteria for critical selection [16]; ** No published value.

The comparison of this data (Table 9-3) shows that no linear relationship between the ligand basicity and the values of the stability constants occurs. For instance, even though BIS-TRIS has a lower $\text{p}K_a$ value than TAPS, the $\log K_{\text{ML}}$ value for both metals is higher than for TAPS. The observation of the structures of the different ligands points out to primary coordination by the amine group. The variation of the number of hydroxo groups in the ligands is possibly the explanation for the fact that BIS-TRIS has a high $\log K_{\text{ML}}$ value, even with a low $\text{p}K_a$. Since this ligand has the higher number of hydroxo groups, it is reasonable to consider the hypothesis of coordination also by this donor atom.

According to Pearson's classification of hard and soft behavior [20], cadmium is a soft acid and zinc is borderline acid; soft metal ions prefer ligands with soft donor atoms (*e.g.* R_3N , RSH), and hard metal ions prefer ligands with hard donor atoms (*e.g.* OH , ROH , RNH_2). This indicates that zinc, besides binding with the amine groups, can also bind with the OH group.

The relationship between $\log K_{\text{ML}}$ and $\text{p}K_a$ for Cd^{2+} and Zn^{2+} 1:1 complexes of BIS-TRIS, TES, TAPSO, TRIS, TAPS, AMPSO, Ammonia, Ethanolamine and Methylamine was plotted in Figure 9-6.

It is evident that the data for zinc can fit rather well to a straight line (Figure 9-6B), indicating that $\log K_{\text{ZnL}}$ increases with the $\text{p}K_a$. This is not so obvious for cadmium (Figure 9-6A) where the stability constants values appear to be independent of the basicity of the ligand considered (within their error limits). The straight line observed for Zn^{2+} systems indicates a coordination of the metal to the ligand by the amine group. In addition, the data pairs $\text{Zn}(\text{TAPSO})/\text{HTAPSO}$ and $\text{Zn}(\text{BIS-TRIS})/\text{HBIS-TRIS}$ are above the reference line demonstrating an increasing of the complex stability. Hence, a further interaction aside from the one with the amine group must take place; this can only be due to a bond to a hydroxo group, as stated before. The vertical distance (dashed line in Figure 9-6B) between the data point and its reference line reflects probably the formation of a five-membered chelate ring.

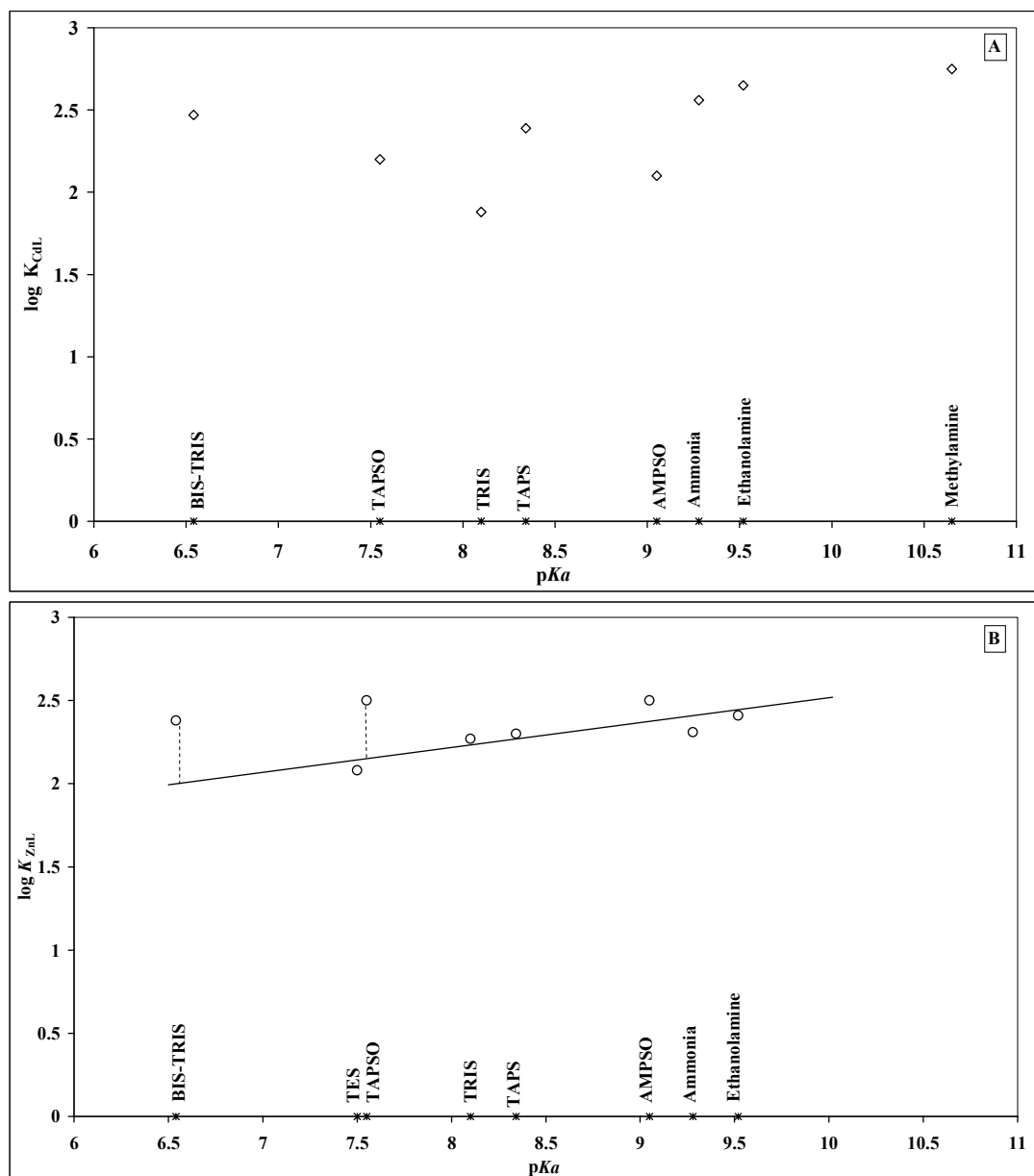


Figure 9-6 Relationship between $\log K_{ML}$ and pK_a for Cd^{2+} (A) and Zn^{2+} (B) 1:1 complexes of several biological buffers based on the results given in Table 9-3.

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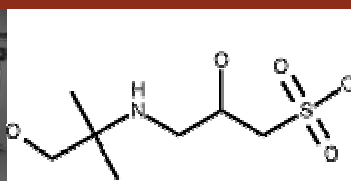
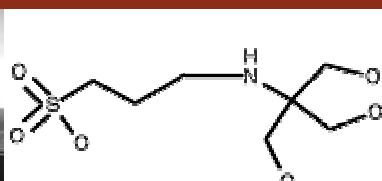
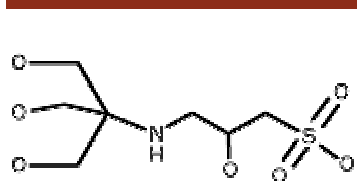
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PART V MODELLING OF $\text{Ni-(BUFFER)}_x\text{-(OH)}_y$ AND $\text{Co-(BUFFER)}_x\text{-(OH)}_y$ SYSTEMS

10 COMPLEXATION OF COBALT AND NICKEL IONS BY SOME ZWITTERIONIC BIOLOGICAL BUFFERS IN AQUEOUS SOLUTION: A POTENTIOMETRIC AND SPECTROMETRIC STUDY⁹

ABSTRACT

Cobalt(II) and nickel(II) complexation properties with three zwitterionic biological buffers, TAPSO, TAPS and AMPSO, were evaluated by GEP and UV-Vis spectroscopy, at 25.0 °C and 0.1 M KNO₃ ionic strength. These buffers have pK_a values from 7.55 to 9.05, which allow them to be used in biological systems. The study showed that all the three buffers complex cobalt(II) and nickel(II) ions. For Ni-(TAPSO)_x-(OH)_y and Co-(TAPSO)_x-(OH)_y systems, the proposed final models and overall stability constants were obtained by combining results from both techniques. The values for the stability constants for the Ni-(TAPSO)_x-(OH)_y system are: $\log \beta_{\text{NiL}} = 2.99 \pm 0.06$ and $\log \beta_{\text{NiL2}} = 4.8 \pm 0.1$. The overall stability constants for the Co-(TAPSO)_x-(OH)_y system are: $\log \beta_{\text{CoL}} = 2.2 \pm 0.1$, $\log \beta_{\text{CoL2}} = 3.6 \pm 0.2$ and $\log \beta_{\text{CoL(OH)}} = 7.6 \pm 0.1$, being this last species characterized by a major structural arrangement. For TAPS and AMPSO, the definition of the M-(L)_x-(OH)_y systems, where L = TAPS or AMPSO, in the buffering pH-range was not possible due to the precipitation of the metal hydroxide species. Nevertheless, the obtained results clearly indicate complexation and warn users about the use of these buffers in speciation studies that have nickel or cobalt in solution.

10.1 INTRODUCTION

Buffers are compounds that undergo reversible protonation and, thus aid in maintaining the pH of a solution. This is particularly important in biological systems, which are often sensitive to small changes in pH.

For the standardization of pH and control of acidity in the physiological region of pH 7 to 9, Good *et. al.* [1] and Ferguson *et. al.* [2] have listed hydrogen buffers, which are N-substituted amino sulfonic acids compatible with common biological media. These buffers have properties that generally make their usage advantageous for experiments with cellular systems and macromolecules. However, several of these buffers demonstrated complexing capabilities with cations [3] and can therefore inhibit a series of metal ion-dependent biochemical reactions. Albeit these pH buffers tend to bind metal ions much more weakly than do strong chelators or proteins, the pH buffer is usually present in

⁹ To be submitted for publication

significant excess and hence can have an appreciable effect on metal speciation in solution. In fact, there has been an increase in reports on buffer interferences when most of Good's buffers were used in the presence of metal ions [4, 5]; many conflicting data and conclusions that were reported by investigators studying identical metal-cation protein systems (at the same pH in carefully executed experiments) might be due in part to the failure to include the presence of metal-buffer complexes [6]. The three biological buffers studied in this work, TAPSO, TAPS and AMPSO, are structural related and contain at least two hydroxyl groups in β carbons nearby to a secondary amino group (Figure 10-1). Specifically, in the case of TAPSO and TAPS, the only difference between these two buffers is the fact that TAPSO has one more hydroxyl group than TAPS in the β carbon nearby the sulfonic group.

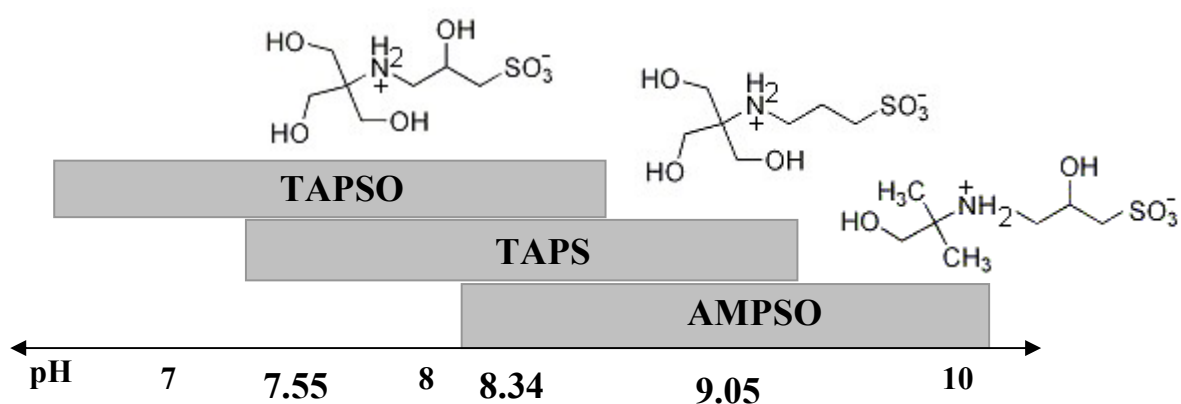


Figure 10-1 Structures, pK_a values and pH range of the buffers tested in this work.

For TAPS, an exhaustive speciation study of $M^{2+}-(TAPS)_x-(OH)_y$, with $M^{2+} = Ni^{2+}$, has never been performed and the reported formation constants between cobalt and TAPS [7], cobalt or nickel and AMPSO [8] or TAPSO [9-11] do not meet the critical selection defined by Martell and Smith [3, 12]. In this context, we think that the study of the interaction of TAPSO, TAPS and AMPSO with two important biological metals, nickel(II) and cobalt(II), may furnish a further contribution to obtain a systematic information of the overall stability constants of the (Good buffer)-(metal ion) systems where complexation occurs.

In the present work, the interaction between the three buffers (TAPSO, TAPS and AMPSO) and nickel or cobalt was explored using pH-potentiometric and UV/Vis spectroscopic techniques, at fixed total ligand to total metal ion concentration ($[L_T]:[M_T]$) ratios and various pH values, at 25.0 °C and 0.1 M KNO₃ ionic strength.

10.2 EXPERIMENTAL

10.2.1 MATERIALS AND REAGENTS

Merck analytical grade nitrate salts of the metals, $\text{Ni}(\text{NO}_3)_2$ and $\text{Co}(\text{NO}_3)_2$, were used, and their purity was determined by complexometric ethylenediaminetetraacetate (EDTA) titration [13].

Additional information about the materials and reagents used, were described previously (Part II: Chapter 2, Section 2.2.1, Chapter 3, Section 3.2.1 and Chapter 4, Section 4.2.1).

10.2.2 APPARATUS

10.2.2.1 POTENTIOMETRY

Potentiometric titrations of $\text{Ni}-(\text{L})_x-(\text{OH})_y$ and $\text{Co}-(\text{L})_x-(\text{OH})_y$ systems ($\text{L} = \text{TAPSO}$, TAPS and AMPSO) were performed as described previously (Part II, Chapter 2, Section 2.2.2.2).

10.2.2.2 UV-VIS SPECTROSCOPY

The UV-Vis absorption spectra for $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ and $\text{Co}-(\text{TAPSO})_x-(\text{OH})_y$ systems were recorded at $25.0\text{ }^\circ\text{C}$ on a UV-Vis 8500 double-beam spectrophotometer from Techcomp (Hong Kong), digitally (1 nm) over the range 190 – 1100 nm, using standard 1-cm quartz cells.

10.2.3 PROCEDURE

10.2.3.1 CALIBRATION OF THE GLASS ELECTRODE

The calibration of the glass electrodes (pH measured as $-\log [\text{H}^+]$) was accomplished as previously described (Part II, Chapter 3, Section 3.2.3.1).

10.2.3.2 GLASS ELECTRODE POTENTIOMETRY

The measurements reported in this work were performed on solutions adjusted to ionic strength 0.1 M KNO_3 in a Metrohm (Herisau, Switzerland) jacketed glass vessel, equipped with a magnetic stirrer, and thermostatted at $25.0 \pm 0.1\text{ }^\circ\text{C}$ using a water bath.

For characterization of Ni-(TAPSO)_x-(OH)_y system, three [L_T]:[Ni_T] ratios were studied: 2.0, 4.7 and 6.1, with [Ni²⁺] = 5.0×10^{-3} , 1.5×10^{-2} and 4.9×10^{-3} M, respectively. For each ratio, two independent solutions were prepared and two titrations of each solution were performed. For Ni-(TAPS)_x-(OH)_y system, four titrations using two independent solutions were carried out for the two ratios studied: [L_T]:[Ni_T] ratio 6.2, with [Ni²⁺] = 4.9×10^{-3} M and [L_T]:[Ni_T] ratio 15.0, with [Ni²⁺] = 5.0×10^{-3} M. For Ni-(AMPSO)_x-(OH)_y system, two [L_T]:[Ni_T] ratios were studied: 6.0 and 8.5 with [Ni²⁺] = 8.3×10^{-3} and 6.0×10^{-3} M, respectively.

For Co-(TAPSO)_x-(OH)_y system, eight titrations of different ratios were carried out. The [L_T]:[Co_T] ratios studied were: 2.0, 4.6 and 5.9 with a total cobalt concentration of 5.0×10^{-3} , 1.5×10^{-2} and 5.1×10^{-3} M, respectively. For the other systems, only one ratio was analysed for each one: [TAPS_T]:[Co_T] = 10.0, [Co²⁺] = 5.2×10^{-3} M and [AMPSO_T]:[Co_T] = 17.0, [Co²⁺] = 5.3×10^{-3} M; for each system, four titrations of two different solutions were performed.

For all systems, monotonic additions of standardised KOH were done and the potential was recorded as a function of the added volume.

10.2.3.3 UV-VIS SPECTROSCOPY

The Beer-Lambert law was obeyed for metals ions solutions under the experimental conditions used (data not shown): $0.005 < [\text{Ni}^{2+}] < 0.03\text{M}$ and $0.01 < [\text{Co}^{2+}] < 0.04\text{M}$.

For Ni-(TAPSO)_x-(OH)_y system, two ratios were studied, [L_T]:[Ni_T] ratio 2.0, with [Ni²⁺] = 3.0×10^{-2} M and [L_T]:[Ni_T] ratio 3.8, with [Ni²⁺] = 1.6×10^{-2} M. For both ratios, the spectrum was scanned from 500 to 1100nm. For [L_T]:[Ni_T] ratio 2.0, fifteen batch solutions were prepared in the pH range between 3.0 and 7.6 and for [L_T]:[Ni_T] ratio 3.8, fourteen batch solutions were prepared between pH 4.4 and 8.0.

For spectroscopic analysis of Co-(TAPSO)_x-(OH)_y system, [L_T]:[Co_T] ratios 1.7 and 5.2, with [Co²⁺] = 3.0×10^{-2} and 1.5×10^{-2} M, respectively, were carried out. For both ratios, the spectrum was scanned from 300 to 1100nm. For [L_T]:[Co_T] ratio 1.7, twelve batch solutions were prepared in the pH range between 5.6 and 7.5 and for [L_T]:[Ni_T] ratio 5.2, fifteen batch solutions were prepared between pH 5.0 and 7.9.

The titration of each system was performed in the batch form. A stock solution for each ratio of each system was prepared. The pH of the batch solutions was changed by adding very small additions of a very concentrated nitric acid solution and/or saturated KOH solution with negligible variation of the total volume, and measured with a combined Crison glass electrode (reference electrode Ag/AgCl).

10.2.4 DATA TREATMENT

During the refinement of the overall stability constants for both systems and for both techniques, the water [14] (Part II, Chapter 2, Table 2-1) and ligands (Part II: Chapter 2, Table 2-1; Chapter 3, Table 3-1; and Chapter 4, Table 4-1) protonation constants, as well as all known stability constants for $\text{M}_x(\text{OH})_y$ species [14] (Table 10-1), were kept fixed.

Table 10-1 Overall stability constants for Ni^{2+} and Co^{2+} complexes with OH^- , at 25.0 °C [14].

	Equilibrium	Log β	I (M)
Nickel	$\text{Ni}^{2+} + \text{OH}^- \rightleftharpoons \text{Ni}(\text{OH})^+$	3.6	0.1
	$2\text{Ni}^{2+} + \text{OH}^- \rightleftharpoons \text{Ni}_2(\text{OH})^{3+}$	4.7	1.0
	$\text{Ni}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Ni}(\text{OH})_2$	9.0	0.0
	$\text{Ni}^{2+} + 3\text{OH}^- \rightleftharpoons \text{Ni}(\text{OH})_3^-$	12.0	0.0
	$4\text{Ni}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Ni}_4(\text{OH})_4^{4+}$	28.3	0.0
	$\text{Ni}(\text{OH})_2 (\text{s}) \rightleftharpoons \text{Ni}^{2+} + 2\text{OH}^-$	-15.2	0.0
Cobalt	$\text{Co}^{2+} + \text{OH}^- \rightleftharpoons \text{Co}(\text{OH})^+$	4.3	0.0
	$2\text{Co}^{2+} + \text{OH}^- \rightleftharpoons \text{Co}_2(\text{OH})^{3+}$	3.0	0.0
	$\text{Co}^{2+} + 2\text{OH}^- \rightleftharpoons \text{Co}(\text{OH})_2$	9.2	0.0
	$\text{Co}^{2+} + 3\text{OH}^- \rightleftharpoons \text{Co}(\text{OH})_3^-$	10.5	0.0
	$\text{Co}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Co}(\text{OH})_4^{2-}$	9.7	0.0
	$4\text{Co}^{2+} + 4\text{OH}^- \rightleftharpoons \text{Co}_4(\text{OH})_4^{4+}$	25.5	0.0
	$\text{Co}(\text{OH})_2 (\text{s}) \rightleftharpoons \text{Co}^{2+} + 2\text{OH}^-$	-14.9	0.0

10.2.4.1 GLASS ELECTRODE POTENTIOMETRY

The simulation and optimization procedures of potentiometric data was done using ESTA program [15, 16].

The refinement operations used in potentiometry involve solving mass-balance equations, including equation for the total proton concentration, in such a way that the computed free proton concentration, when used by the equation describing the response of the calibrated glass electrode, reproduces the experimentally recorded potential of the glass electrode as accurately as possible. This is done by minimization of an objective function, U , defined as:

$$U = (N - n_p)^{-1} \sum_{n=1}^N (E_n^{obs} - E_n^{calc})^2 \quad (10-1)$$

where U is the objective function to be minimized, N is the total number of experimental titration points; n_p represents the number of parameters simultaneously optimized, E_n^{obs} observed electrode potential at the n^{th} data point and $E_n^{calc} = E^0 + k \log[H^+]$, E^0 is the electrode intercept and k the electrode calibration slope. A Gauss-Newton method is the adopted approach by ESTA to minimize U .

Hamilton R-factor, R^H , is the statistical parameter used by ESTA to reflect the improved agreement between calculated and observed data. It is given by:

$$R^H = \left[\frac{U}{\sum_{n=1}^N (E_n^{obs})^2} \right]^{1/2} \quad (10-2)$$

Hamilton R-factor does not have dimensions.

ESTA software includes a metal formation function, Z_M , defined by:

$$Z_M = \frac{[L_T] - L \left(1 + \sum_{n=1}^N \beta_{n01} [H^+]^n \right)}{[M_T]} \quad (10-3)$$

where

$$L = \frac{[H_T] - [H^+] + [OH^-]}{\sum_{n=1}^N \beta_{n01} [H^+]^n} \quad (10-4)$$

$[L_T]$, $[M_T]$ and $[H_T]$ represent the total concentration of ligand, metal and proton, respectively; β_{n01} stands for the formation constants of $M_nL_0H_1$ species. This function is calculated for each datum point and is plotted against $-\log[L]$ to aid in the modelling procedures.

It is important to stress that the Z_M function can only be generated for the assumed $M-(L)_x-(OH)_y$ model. This implies that one has to investigate numerous possible models and generate Z_M functions for all of them in order to select most probable one, usually based on the observed fit of the theoretical function into the objective (experimental) one.

10.2.4.2 UV-VIS SPECTROSCOPY

The refinement of the UV-Vis data was performed using the program pHab [17]. This program is concerned with the determination of equilibrium constants using absorbance data obtained from solutions at fixed pH. The chemical solutions are independent of each other and therefore the data is said to consist of single data points, i.e. measurement of an absorbance spectrum for a single solution of known composition. The known composition is specified in terms of the analytical concentrations of all the reagents present in the mixture, except one: for that one the "free" concentration is given in place of the analytical concentration. Most commonly the known free concentration will be related to the hydrogen ion and the concentration value will be expressed as pH. Each species present in solution may be either colourless or coloured. For each coloured species, the molar absorptivity may be known or unknown (refinable).

In the refinement procedures, the concentration $[H^+]$ is taken from the observed pH (input data). The free metal $[M]$ and free ligand $[L]$ concentrations are obtained from the mass-balance equations, knowing the stability constants $\beta_{M_p L_q OH_r}$ (charges omitted for sake of simplicity)

$$[M_T] = [M] + \sum_{p=1} \sum_{q=1} \sum_{r=0} p \beta_{M_p L_q OH_r} [M]^p [L]^q [OH]^r \quad (10-5)$$

$$[L_T] = [L] + \sum_{p=1} \sum_{q=1} \sum_{r=0} q \beta_{M_p L_q OH_r} [M]^p [L]^q [OH]^r \quad (10-6)$$

where $\beta_{M_p L_q OH_r}$ stand for the overall stability, $[M]$, $[L]$, and $[OH]$ stand for the free metal ion, free ligand, and hydroxide concentration, respectively. In case of the formation of complexes involving the protonated form of a ligand, *e.g.* MLH, the term $[M]^p [L]^q [OH]^r$ would be replaced by $[M]^p [L]^q [H]^r$.

Having solved the equations for $[M]$ and $[L]$ and knowing $[H]$ from the observed pH all the species' concentrations can be derived and hence, through Beer's law, all the molar absorbance.

$$A_{s\lambda}^{calc} = l_s \sum_{i=1-n} C_{si} \epsilon_{i\lambda} \quad (10-7)$$

In Equation (10-7), $A_{s\lambda}^{calc}$ represents the calculated absorbance of a solution *s* at a wavelength λ . The term l_s is the optical path-length used for the solution *s*, C_{si} stands for the concentration of the *i*th species in solution *s* and $\epsilon_{i\lambda}$ the molar absorptivity of the *i*th species at the wavelength λ . Optimising the agreement between observed and calculated absorbance refines the stability constants values. The standard deviation, σ , reflects the improved agreement between calculated and observed data.

10.3 RESULTS AND DISCUSSION

The three biological buffers under study in this work (TAPSO, TAPS and AMPSO) are structural related (Figure 10-1). Regarding the basicity of the amino group, it is possible to verify that the substitution of hydroxo groups in the ligand leads to a systematic decrease of the pK_a values: AMPSO, TAPS and TAPSO have two, three and four hydroxyl groups, respectively, and their pK_a values are 9.05, 8.34 and 7.55, respectively (Table 10-1). As a consequence, these buffers will deprotonate at pH values progressively more basic, which will influence their metal complexation behaviour.

10.3.1 MODELLING OF $M^{2+}-(TAPSO)_x-(OH)_y$ SYSTEMS AND REFINEMENT OF STABILITY CONSTANTS

10.3.1.1 $M^{2+} = Ni^{2+}$

Glass electrode potentiometric titrations were performed to determine the extension of the interaction between Ni and TAPSO. Three ligand total to total metal concentration ratios ($[L_T]:[Ni_T]$) were performed 2.0, 4.7 and 6.1. The final results are summarized in Table 10-2. For the lowest $[L_T]:[Ni_T]$ ratio, $[L_T]:[Ni_T] = 2.0$ with $[Ni_T] = 5.0 \times 10^{-3}$ M, NiL complex was the only species, that could be refined (Model 1, Table 10-2) before precipitation occurrence, around pH 7.3. For $[L_T]:[Ni_T]$ ratio of 6.1, besides the refined stability constants for the three models tested, which were incorporated in Table 10-2, the graphical analysis of Z_M was also included in Figure 10-2A. To make the figure simpler, results of only one titration were included; results for the others titrations were similar. The refinement operations started with the fit of the simplest model, just NiL species, and as one can see the model did not fit well the experimental results; the calculated Z_M function for higher pH values (lower $-\log[L]$ values) was below the experimental Z_M function indicating that other(s) species was(were) missing. Since the function grows above 1, NiL_2 can be the species necessary to complete the model [18]. As one can see in Table 10-2 and Figure 10-2A, this model (Model 2 in Table 10-2) fitted well the experimental data and an improvement in the Hamilton R-factor was observed. Although the Z_M function did not present any curve-back shape [18], which would indicate the presence of hydroxide species, it is possible that some amount of $NiL(OH)$ species was formed. However the fit of this model, $NiL + NiL(OH)$ (Model 3 in Table 10-2), did not improve the results and a worst Hamilton R-factor than that obtained with Model 2 was calculated. In addition, the refined stability constant for $NiL(OH)$ species has an unreal value (about 2.4 log units above the theoretical one). If one considers that the formation of $NiL(OH)$ results from the hydrolyses of NiL complex, the stability constant for this species should have the following value: $\log \beta_{NiL(OH)} = \log \beta_{NiL} + \log \beta_{Ni(OH)} = 3.04 + 3.6 = 6.64$. Such a huge increase would mean that the formation of $NiL(OH)$ resulted from a major structural change of the complex, which is most unlikely.

Table 10-2 Overall stability constants (as $\log \beta$ values) for $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ system determined at 25.0 °C by GEP for $[\text{L}_T]:[\text{Ni}_T] = 2.0$ (two titrations, 219 points, $\text{pH}=5.4 - 7.0$), 4.7 (three titrations, 910 points, $\text{pH} = 4.4 - 7.6$) and 6.1 (two titrations, 476 points, $\text{pH} = 4.0 - 7.3$), with $[\text{Ni}^{2+}] = 5.0 \times 10^{-3}$, 1.5×10^{-2} and $4.9 \times 10^{-3}\text{M}$, respectively.

Complexes	$[\text{L}_T] : [\text{Ni}_T]$	2.0			4.7			6.1		
		Model 1	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
NiL^+ $\text{Ni}^{2+} + \text{L}^- \rightleftharpoons \text{NiL}^+$		2.9215±0.0006	3.00±0.01	3.050±0.002	3.096±0.003	3.048±0.004	3.077±0.004	3.0185±0.0008	3.0389±0.0009	3.025±0.001
NiL_2 $\text{Ni}^{2+} + 2\text{L}^- \rightleftharpoons \text{NiL}_2$		NI	NI	4.792±0.005	NI	4.654±0.008	NI	4.676±0.003	NI	4.50±0.01
$\text{NiL}(\text{OH})$ $\text{Ni}^{2+} + \text{L}^- + \text{OH}^- \rightleftharpoons \text{NiL}(\text{OH})$		NI	NI	NI	9.367±0.007	8.73±0.02	NI	NI	9.051±0.003	8.57±0.02
Hamilton R-factor		0.01518	0.1207	0.03060	0.04342	0.02595	0.05271	0.00847	0.01047	0.00739

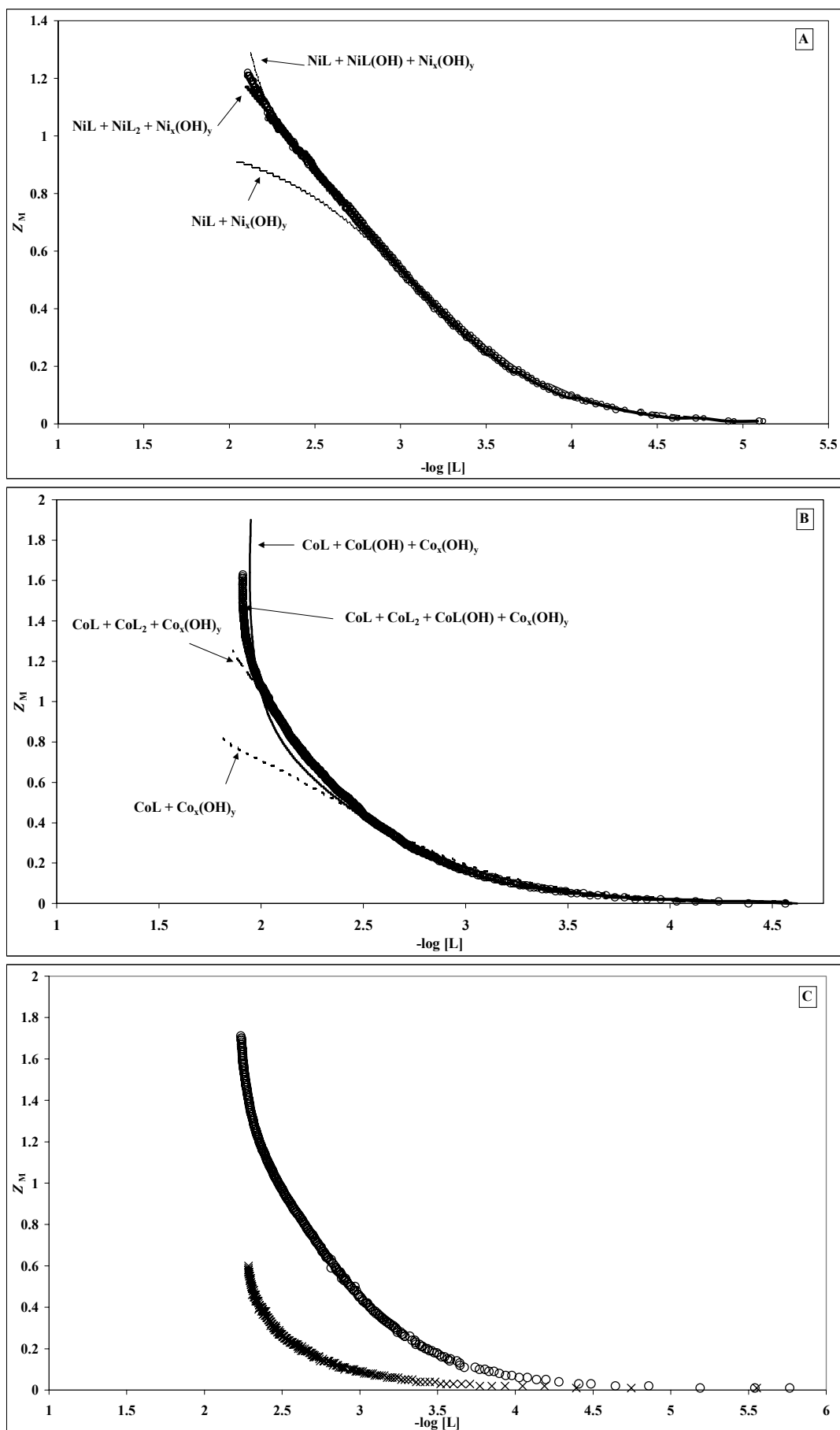
Hamilton R-factor stands for a statistical parameter generated by the program ESTA (see Part V, Chapter 10, Section 10.2.4.1). NI – not included.

Figure 10-2A shows that the adjustment of these two models was identical in a large range of pH, being the departure from the experimental curve (points in Figure 10-2A) only occurred at high pH values. Figure 10-2A suggests that Model 2 reproduces better the experimental data than Model 3. The next logical step would be the inclusion in the model of the three species, NiL , NiL_2 and $\text{NiL}(\text{OH})$. Although the refinement of this model resulted in a better Hamilton R-factor, the refined stability constant value for $\text{NiL}(\text{OH})$ species did not decrease to a realistic value (Table 10-2, Model 4). In order to favour the formation of $\text{NiL}_x(\text{OH})_y$ species, if these species were formed, it was decided to perform another $[\text{L}_\text{T}]:[\text{Ni}_\text{T}]$ ratio, $[\text{L}_\text{T}]:[\text{Ni}_\text{T}] = 4.7$, using a higher metal concentration, $[\text{Ni}^{2+}] = 1.5 \times 10^{-2}$ M. Points were collected up to pH 10.5 but precipitation was observed at about pH 7.6. However similar results to the other ratios were obtained (Table 10-2).

To confirm the species formed for $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ system, a second independent technique, UV-Vis spectroscopy, was used. Compared with potentiometry, spectrometric methods have a higher discriminatory power for the nature of the complex species formed and they are also used for stability constants determination [19].

Two $[\text{L}_\text{T}]:[\text{Ni}_\text{T}]$ ratios were studied: $[\text{L}_\text{T}]:[\text{Ni}_\text{T}]$ ratio 2.0, with $[\text{Ni}^{2+}] = 3.0 \times 10^{-2}$ M and $[\text{L}_\text{T}]:[\text{Ni}_\text{T}]$ ratio 3.8, with $[\text{Ni}^{2+}] = 1.6 \times 10^{-2}$ M. The analysis described below is related to $[\text{L}_\text{T}]:[\text{Ni}_\text{T}] = 2.0$. In Figure 10-3A, the analysed spectrums are presented, where two bands can be identified. Since TAPSO does not absorb in the UV-Vis region, the bands are referred to d-d bands, characteristic of transition metals [20]. According to GEP results, at pH 3.0, no complexation occurs; so, the spectrum recorded at this pH represents the absorption spectrum of the metal aquo-complex in the presence of the ligand but in the absence of complexation. Increasing the pH, an increase of the absorbance occurred and this could only be attributed to the formation of metal complex species, $\text{NiL}_x(\text{OH})_y$, where $x, y = 0, 1$ or 2 (Figure 10-3A).

Figure 10-2 Results from refinement and optimization operations obtained from GEP data. Observed → (circles) and calculated (lines) Z_M functions obtained for: A) $[\text{TAPSO}_\text{T}]:[\text{Ni}_\text{T}] = 6.1$, $[\text{Ni}^{2+}] = 4.9 \times 10^{-3}$ M, pH = 4.0 – 7.3; B) $[\text{TAPSO}_\text{T}]:[\text{Co}_\text{T}] = 5.9$, $[\text{Co}^{2+}] = 5.1 \times 10^{-3}$ M, pH = 4.3 – 7.9; C) $[\text{TAPS}_\text{T}]:[\text{Ni}_\text{T}] = 6.2$ (circles), $[\text{Ni}^{2+}] = 4.9 \times 10^{-3}$ M, between pH = 4.1 and 7.8, and $[\text{AMPSO}_\text{T}]:[\text{Ni}_\text{T}] = 8.5$ (crosses), $[\text{Ni}^{2+}] = 6.0 \times 10^{-3}$ M, between pH = 4.3 and 8.0. The fitted models for $\text{M}-(\text{TAPSO})_x-(\text{OH})_y$ systems are indicated in the figure and the stability constant values are in Table 10-2 and Table 10-4, for nickel(II) and cobalt(II), respectively.



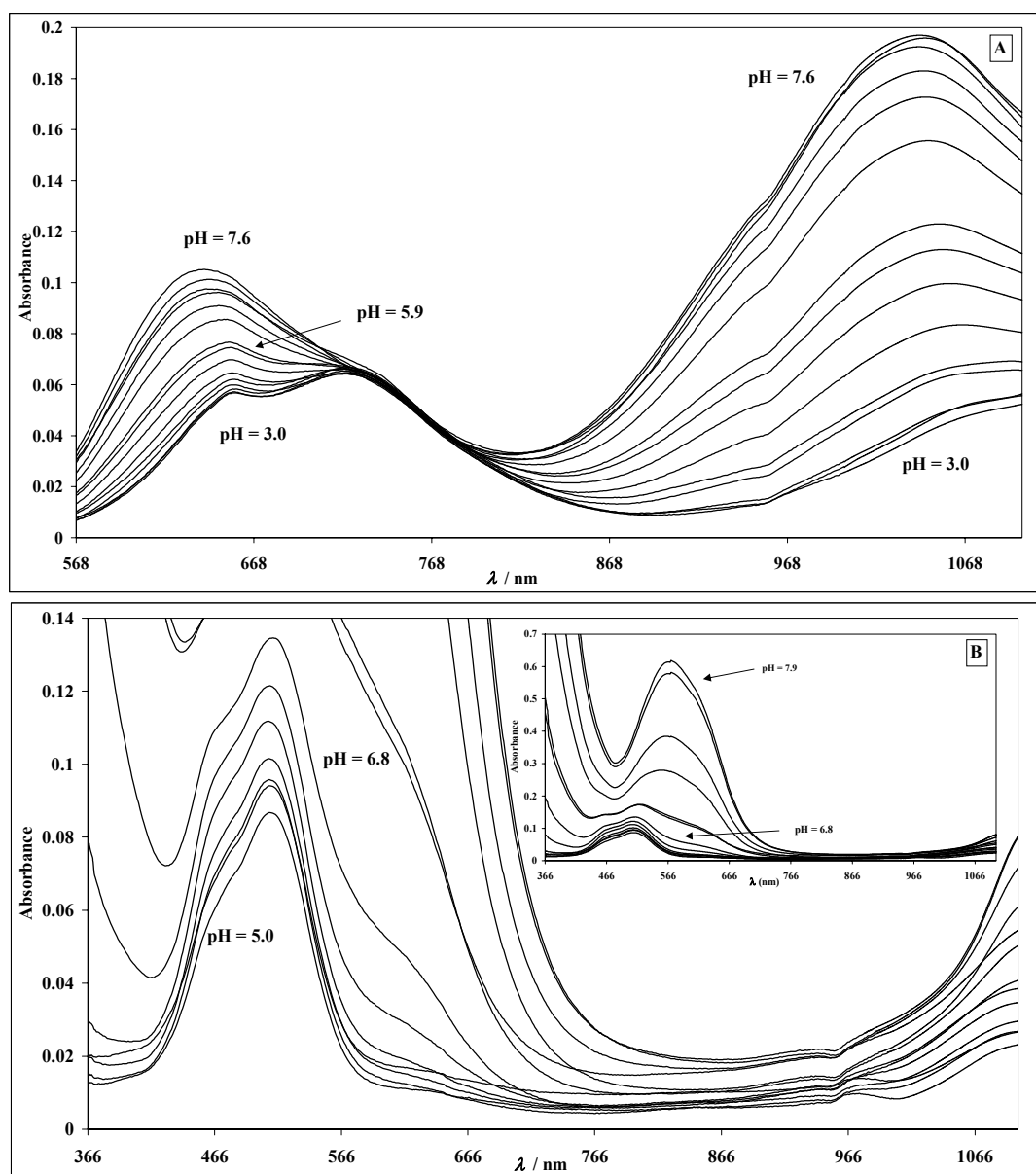


Figure 10-3 Spectrophotometric curves of the titrations: (A) $[\text{TAPSO}_\text{T}]:[\text{Ni}_\text{T}] = 2.0$, $[\text{Ni}^{2+}] = 3.0 \times 10^{-2}\text{M}$, pH between 3.0 and 7.6; (B) $[\text{TAPSO}_\text{T}]:[\text{Co}_\text{T}] = 5.2$, $[\text{Co}^{2+}] = 1.5 \times 10^{-2}\text{M}$, pH between 5.0 and 7.9.

Figure 10-3A shows that the 1025nm band, corresponding to the lowest energy, shifts to lower λ values with the increase of the pH. This indicates a change of the Ni–O bond (O of the water) to Ni–N bond (N of the ligand), which can be due to the presence of NiL species in solution, as it was already demonstrated by GEP results. At the end of the titration, corresponding to the higher pH values, the two bands were identical, which evidences that the same species are present in solution or the existing species have the same ϵ . Stability constants values were refined using the pHab software and the results are compiled in Table 10-3. The refinement operation started with the fit of the model NiL into the experimental data (Model 1, Table 10-3).

Table 10-3 Overall stability constants (as log β values) for Ni-(TAPSO)_x-(OH)_y system determined at 25.0 °C by UV-Vis spectroscopy for $[L_T]:[Ni_T] = 2.0$, $[Ni^{2+}] = 3.0 \times 10^{-2}$ M and $[L_T]:[Ni_T] = 3.8$, $[Ni^{2+}] = 1.6 \times 10^{-2}$ M. For each ratio, 14 batch solutions, spectrums ($568 < \lambda < 1100$ nm) were collected between pH 3.7 to 7.6 and pH 4.4 and 8.0, respectively.

Complexes [L_T]:[Ni_T]	2.0		3.8	
	Model 1	Model 2	Model 1	Model 2
NiL⁺ Ni²⁺ + L⁻ ⇌ NiL⁺	2.971±0.003	2.976±0.004	2.911±0.002	2.938±0.002
NiL₂ Ni²⁺ + 2L⁻ ⇌ NiL₂	NI	4.83±0.09	NI	4.81±0.02
NiL(OH) Ni²⁺ + L⁻ + OH⁻ ⇌ NiL(OH)	NI	NI	NI	R
σ	0.03896	0.03755	0.02054	0.01960

NI – not included.

A refined stability constant value, $\log \beta = 2.971 \pm 0.003$, of similar magnitude to those refined by GEP (Table 10-2) was obtained. Next and because the GEP data (Figure 10-2A and Table 10-2) suggested the presence of a second absorbing species in solution, we decided to include NiL₂ species in the model (Model 2, Table 10-3). For making the refinement operations simpler, the adopted strategy was to calculate previously the molar absorptivity for the NiL complex. For this purpose, calculation was done in the pH range where this complex was the main species present in solution. SDD were simulated using the GEP refined stability constants values (Model 2, Table 10-2). SDD (data not shown) showed that, for the experimental conditions used in UV-Vis, NiL should be the main species present in the pH range between 3.0 and 5.9; thus, data points collected in this pH range were used to calculate the molar absorptivity of NiL species. In addition and since the d-d bands are characteristic of low absorbance bands and no pronounced spectrum variation was observed throughout the pH range analysed (Figure 10-3A), which evidences that no major structural arrangement of the complexes should be expected, one can assume that the molar absorptivity of the NiL and NiL₂ species formed in solution are identical [21]. Thus, in the refinement operations, equal molar absorptivity values for NiL and NiL₂ were assumed for both ratios. Table 10-3 evidences that, for both ratios tested by UV-Vis, the refined values for $\log \beta_{NiL}$ and $\log \beta_{NiL_2}$ were equivalent and a slight improvement of the overall fit suggest that these two species should be present in solution. It is important to mention that the same behaviour occurred by GEP (Table 10-2). Additionally, the presence of a third species, NiL(OH), was tested and the refinement operations confirmed that no other species, besides NiL and NiL₂, were present. It is important to mention that the value of NiL(OH) stability constant, refined by

GEP, was two orders of magnitude higher than the theoretical one; if this species was formed, this should result in a change of the complex geometry, which was not detected by UV-Vis (Figure 10-3A).

The comparative analysis of the stability constant values obtained for Model 2 by both techniques (Table 10-2 and Table 10-3) shows that they are identical within the experimental errors of the techniques. The final model and overall stability constants after considering all the experimental results are: NiL and NiL₂, with overall stability constants set to (as $\log \beta$) 3.00 ± 0.06 and 4.8 ± 0.1 . Note that the standard deviations indicate here the ranges in stability constants obtained for the same model refined from several independent titrations by two independent techniques.

SDD were drawn for some [L_T]:[Ni_T] ratios (Figure 10-4). For [L_T]:[Ni_T] = 2.0 ([Ni²⁺] = 5.0×10^{-3} M) used in GEP, NiL is the main species present in solution up to pH 7.0; only 5% of NiL₂ is predicted at this pH (Figure 10-4A), which is a small amount to be detected by ESTA. This fact corroborates the impossibility of refining NiL₂ for this [L_T]:[Ni_T] ratio (Table 10-2). For [L_T]:[Ni_T] = 4.7 ([Ni²⁺] = 1.5×10^{-2} M) (Figure 10-4B) and 6.1 ([Ni²⁺] = 4.9×10^{-3} M) (data not shown), SDD clearly showed that NiL and NiL₂ species are formed under the experimental conditions tested; at pH 7.3, an appreciable amount of NiL₂ (about 50 and 30%, respectively) was predicted in solution for both [L_T]:[Ni_T] ratios. For experimental conditions tested by UV-Vis, SDD (Figure 10-4C and D) evidenced that, for both [L_T]:[Ni_T] ratios, NiL and NiL₂ species were formed, being predicted an appreciable amount of NiL₂ (at pH 7.5, about 30 and 50% for [L_T]:[Ni_T] = 2.0 and 3.8, respectively). Additionally, for [L_T]:[Ni_T] = 2.0, SDD evidenced that, up to pH 5.9, NiL was the main species in solution, which means that the experimental conditions used for calculating the molar absorptivity of this complex were gathered.

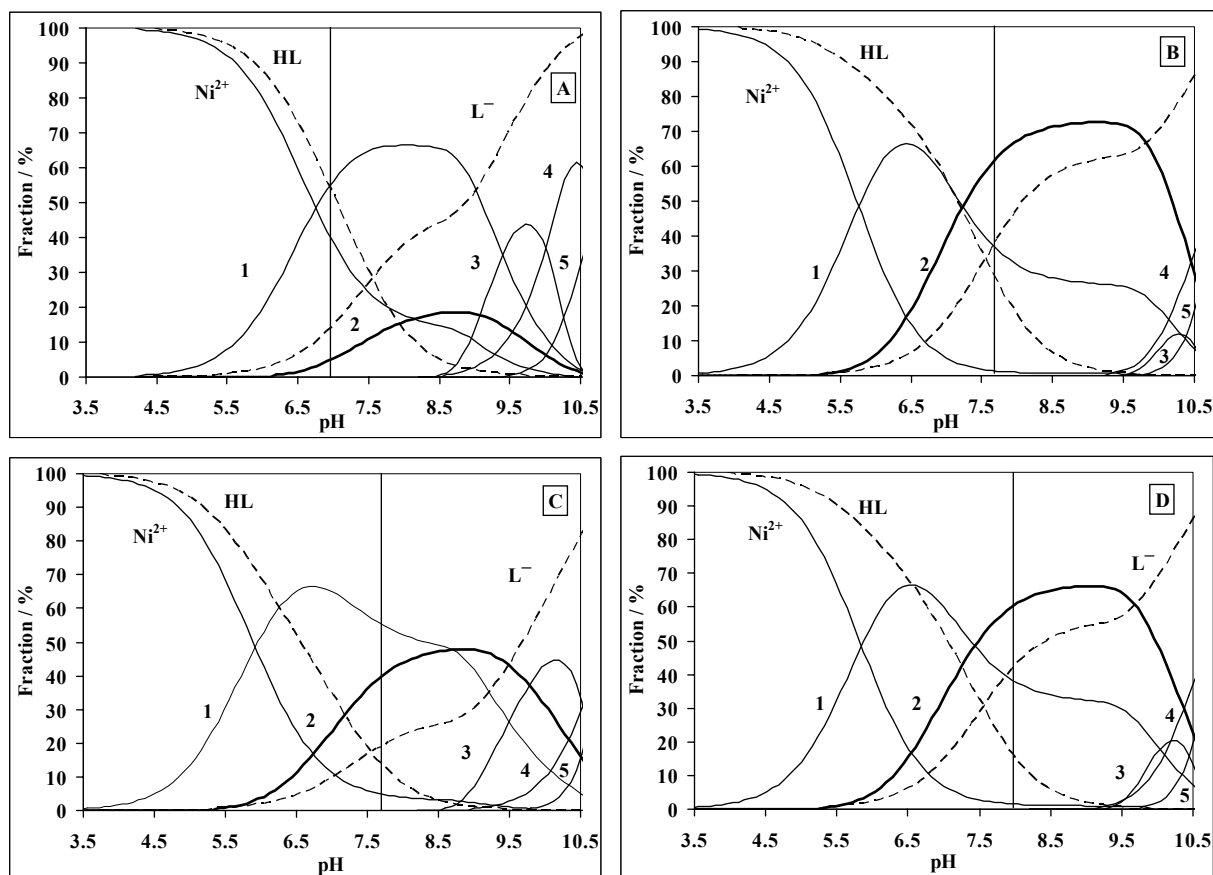


Figure 10-4 Species distribution diagrams plotted for the final model for $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ system: $\log \beta_{\text{NiL}} = 3.0$ and $\log \beta_{\text{NiL}_2} = 4.8$. A and B correspond to GEP conditions: $[\text{L}_\text{T}] : [\text{Ni}_\text{T}] = 2.0$, $[\text{Ni}^{2+}] = 5.0 \times 10^{-3} \text{ M}$ and $[\text{L}_\text{T}] : [\text{Ni}_\text{T}] = 4.7$, $[\text{Ni}^{2+}] = 1.5 \times 10^{-2} \text{ M}$, respectively. C and D correspond to UV-Vis experiments: $[\text{L}_\text{T}] : [\text{Ni}_\text{T}] = 2.0$, $[\text{Ni}^{2+}] = 3.0 \times 10^{-2} \text{ M}$ and $[\text{L}_\text{T}] : [\text{Ni}_\text{T}] = 3.8$, $[\text{Ni}^{2+}] = 1.6 \times 10^{-2} \text{ M}$, respectively. The species included in the SDD are: 1 – NiL , 2 – NiL_2 , 3– $\text{Ni}_4(\text{OH})_4$, 4 – $\text{Ni}(\text{OH})_2$ and 5 – $\text{Ni}(\text{OH})_3$. Values for $\log \beta_{\text{Ni}_x(\text{OH})_y}$ were from Table 10-1. The vertical line represents the pH at which precipitation of $\text{Ni}(\text{OH})_2$ was predicted.

10.3.1.2 $\text{M}^{2+} = \text{Co}^{2+}$

The characterization of $\text{Co}-(\text{TAPSO})_x-(\text{OH})_y$ system was also based in GEP and UV-Vis data analyses. By potentiometry, three $[\text{L}_\text{T}]:[\text{Co}_\text{T}]$ ratios were studied: 2.0, 4.6 and 5.9 with a $[\text{Co}^{2+}]$ of 5.0×10^{-3} , 1.5×10^{-2} and $5.1 \times 10^{-3} \text{ M}$, respectively. The final results obtained after the refinement operations using various models are summarized in Table 10-4. For the lowest ratio tested, $[\text{L}_\text{T}]:[\text{Co}_\text{T}] = 2.0$, only CoL complex was possible to be refined before precipitation, that occurred around pH 7.3. For $[\text{L}_\text{T}]:[\text{Co}_\text{T}] = 5.9$, points were collected up to pH 7.9 and Z_M calculated for each data point was represented as a function of the $-\log[\text{L}]$ in Figure 10-2B. The analysis of figure shows that the function grows up to 1, indicating the formation of CoL [18], followed by a slight curve shape and a rapid increase of Z_M .

Table 10-4 Overall stability constants (as $\log \beta$ values) for $\text{Co}-(\text{TAPSO})_x-(\text{OH})_y$ system determined at 25.0 °C by GEP for $[\text{L}_T]:[\text{Co}_T] = 2.0$ (four titrations, two independent solutions, 480 points, $\text{pH}=4.8 - 7.3$), 4.6 (two titrations, 324 points, $\text{pH} = 5.6 - 7.6$) and 5.9 (two titrations, 934 points, $\text{pH} = 4.3 - 7.9$), with $[\text{Co}^{2+}] = 5.0 \times 10^{-3}$, 1.5×10^{-2} and $5.1 \times 10^{-3}\text{M}$, respectively.

[L_T] : [Co_T] Complexes	2.0	4.6				5.9			
	Model 1	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
CoL ⁺ Co ²⁺ + L [−] ⇌ CoL ⁺	2.308±0.002	2.274±0.007	2.113±0.002	2.195±0.002	2.144±0.001	2.381±0.005	2.192±0.002	2.320±0.002	2.234±0.001
CoL ₂ Co ²⁺ + 2L [−] ⇌ CoL ₂	NI	NI	3.644±0.004	NI	3.433±0.005	NI	4.070±0.004	NI	3.886±0.003
CoL(OH) Co ²⁺ + L [−] + OH [−] ⇌ CoL(OH)	NI	NI	NI	8.078±0.005	7.676±0.008	NI	NI	8.024±0.005	7.638±0.006
Hamilton R-factor	0.0197	0.0608	0.01076	0.01432	0.00424	0.04071	0.01135	0.01503	0.00488

Hamilton R-factor stands for a statistical parameter generated by the program ESTA (see Section 10.2.4.1). NI – not included.

This latter behaviour suggests that the main species in this pH region should be $\text{CoL}_x(\text{OH})$ species [18]. The formation CoL_2 is not expected to occur in a large amount because Z_M function does not grow linearly up to 2 [18] (Figure 10-2B). Even though the analysis of Figure 10-2B suggests the presence of other species than CoL , we decided firstly to test a simpler model just containing CoL species (Model 1, Table 10-4). Figure 10-2B clearly shows that this model does not describe properly the experimental data points. Next, CoL_2 species was included even if there was not an unequivocal evidence of its existence (Figure 10-2B). The Hamilton R-factor was improved (Model 2, Table 10-4) but the graphical analysis shows that, for $[\text{L}]$ higher than 10^{-2} M, the fitting of this model follows a pattern with slope lower than that recorded for the experimental points (Figure 10-2B). Since the analysis of Z_M function performed previously indicated the possible presence of $\text{CoL}(\text{OH})$ species, as a second species present in solution, Model 3, where CoL plus $\text{CoL}(\text{OH})$ were included, was tested. Figure 10-2B shows that this model fitted better the data, even if the Hamilton R-factor was slightly worst (Table 10-4). However, neither Models 2 nor 3 (Table 10-4 and Figure 10-2B) seems to explain completely the experimental points. The next logical step was to include all the species in the model: CoL , CoL_2 and $\text{CoL}(\text{OH})$ (Model 4, Table 10-4). The refinement of this model improved significantly the Hamilton R-factor and the calculated curve fits perfectly into the experimental data, the line goes exactly through the points (Figure 10-2B). Similar results were obtained for $[\text{L}_T]:[\text{Co}_T]$ ratio 4.6 (Table 10-4). For this ratio, Model 4 is the model that fitted better the experimental data (Table 10-4), being the refined overall stability constants values, unless the stability constant value for CoL_2 , in agreement with those refined for $[\text{L}_T]:[\text{Co}_T]$ ratio 5.9.

Next, spectrophotometric studies were performed to complete the analysis of $\text{Co}-(\text{TAPSO})_x-(\text{OH})_y$ system. Two $[\text{L}_T]:[\text{Co}_T]$ ratios were studied, 1.7 and 5.2, with $[\text{Co}^{2+}] = 3.0 \times 10^{-2}$ and 1.5×10^{-2} M, respectively. For $[\text{L}_T]:[\text{Co}_T]$ ratio 5.2, the spectrum curves recorded in the pH range between pH 5.0 and 7.9 are presented in Figure 10-3B. Since TAPSO does not absorb in the UV-Vis region, the spectrum analysis will be referred to the d-d bands characteristics of cobalt [20]. Taking into account GEP results, SDD simulated for $[\text{L}_T]:[\text{Co}_T]$ ratio 5.2 indicated that no significant complexation was expected to occur up to pH 5.0. So, the spectrum recorded at this pH represents the UV-Vis absorption of the cobalt aquo-complex in the presence of the ligand and will be used, as the reference point, for the analysis of the other scans. Increasing the pH, an augment of the absorbance was detected, which was associated with a changing of the observed bands; these facts point out interaction between cobalt and the ligand. Close to 512nm band, a small band around 460nm can be detected, which increases with the pH. This modification of the bands indicates a progressive replacement of the Co–O bond (O of the water) by Co–N bond (N of the ligand due to the formation of CoL species. At pH values higher than 6.8, a large increase of the absorbance of the 575nm band was observed (insert of Figure 10-3B). In addition, the increase of the molar absorbance was accompanied with a decrease of energy, which is a strong indication that the geometry of the species present in solution changed from octahedral to

tetrahedral [22]. According to GEP data, CoL_2 and $\text{CoL}(\text{OH})$ should be present in solution, being this last species capable of acquiring a tetrahedral geometry. Another important observation was that above pH 6.8, the colour of the solutions changed from pink to dark blue, being the intensity of the blue stronger with the increase of pH, which corroborates the decrease of energy (shift of λ towards higher values) (insert of Figure 10-3B). For the lower $[\text{L}_\text{T}]:[\text{Co}_\text{T}]$ ratio, the same behaviour (data not shown) was also observed even with a lower quantity of the new species formed as the titration finished 0.5 pH units early due to precipitation (for $[\text{L}_\text{T}]:[\text{Co}_\text{T}] = 1.7$, points were collected between pH 5.6 and 7.5). These observations and spectroscopic results strongly suggest a preorganization of the Co^{2+} binding site and indicate a geometric changing from octahedral towards tetrahedral. The higher electron-donating ability of OH^- relative to that of H_2O is responsible for the changes in the spectra. The next step was to refine the stability constants values with pHab program; the final results are summarized in Table 10-5. The data refinement for ratio $[\text{L}_\text{T}]:[\text{Co}_\text{T}]$ 1.7 up to pH 6.8 (prior to the spectrum change) only allowed determining the stability constant value for CoL and the respective molar absorptivity. Refinement of the overall stability constants for CoL_2 and $\text{CoL}(\text{OH})$ was also tried but it resulted in theirs rejection. For this $[\text{L}_\text{T}]:[\text{Co}_\text{T}]$ ratio, the molar absorptivity of CoL was also calculated

Table 10-5 Overall stability constants (as $\log \beta$ values) for $\text{Co}-(\text{TAPSO})_x-(\text{OH})_y$ system determined at 25.0 °C by UV-Vis spectroscopy. $[\text{L}_\text{T}]:[\text{Co}_\text{T}] = 1.7$, $[\text{Co}^{2+}] = 3.0 \times 10^{-2} \text{ M}$; spectrums ($366 < \lambda < 1100\text{nm}$) were collected for 7 batch solutions between pH 5.6 and 6.8. $[\text{L}_\text{T}]:[\text{Co}_\text{T}] = 5.2$, $[\text{Co}^{2+}] = 1.5 \times 10^{-2} \text{ M}$; for the same wavelength range, spectrums ($366 < \lambda < 1100\text{nm}$) were collected for 8 batch solutions, between pH 5.0 and 6.8, for Models 1 and 2 and 7 batch solutions, between pH 7.0 and 7.9, for Model 3.

Complexes	$[\text{L}_\text{T}]:[\text{Co}_\text{T}]$		5.2	
	1.7	Model 1	Model 2	Model 3
CoL^+ $\text{Co}^{2+} + \text{L}^- \rightleftharpoons \text{CoL}^+$	2.49±0.01	2.372±0.009	2.34±0.02	2.3 (F)
CoL_2 $\text{Co}^{2+} + 2\text{L}^- \rightleftharpoons \text{CoL}_2$	R	NI	3.6±0.1	3.6 (F)
$\text{CoL}(\text{OH})$ $\text{Co}^{2+} + \text{L}^- + \text{OH}^- \rightleftharpoons \text{CoL}(\text{OH})$	R	NI	NI	7.48 ± 0.04
σ	0.04288	0.06309	0.06304	0.04648

R – rejected; NI – not included; F – fixed value

For [L_T]:[Co_T] ratio 5.2, the refinement operations started by using all spectrums collected between pH 5.0 and 6.8, prior to solution colour change. The first model tested was Model 1, only CoL (Table 10-5). To help in the refinement procedures, the molar absorptivity values for CoL, calculated from the lower ratio (this species exists in higher amount in [L_T]:[Co_T] ratio 1.7), were included as fixed values. Model 2 (CoL + CoL₂) was also tried and identical molar absorptivity values for CoL and CoL₂ species were considered [21], in a similar way as it was done for Ni-(TAPSO)_x-(OH)_y system. Model 2 fitted with an equivalent standard deviation as Model 1 (Table 10-5) and the obtained stability constant values were very comparable with those refined from GEP titrations (Table 10-4). Next, refinement of the experimental data above pH 6.8, for which a structural changing occurred, was performed using the spectrum curves collected from pH 7.0 to 7.9. For an accurate analysis of the data, the stability constants values of CoL and CoL₂ and the respective molar absorptivity values were included as fixed values, plus CoL(OH) species (Model 3, Table 10-5).

Considering the [L_T]:[Co_T] ratios from both techniques for which the final model (CoL, CoL₂ and CoL(OH)) was refined, the following stability constants values were obtained: $\log \beta_{\text{CoL}} = 2.2 \pm 0.1$, $\log \beta_{\text{CoL}_2} = 3.6 \pm 0.2$ and $\log \beta_{\text{CoL(OH)}} = 7.6 \pm 0.1$.

SDD were drawn for all GEP and UV-Vis experimental conditions used. Some typical examples are presented in Figure 10-5. The overall analysis of SDD corroborates the final model (CoL, CoL₂ and CoL(OH)). Thus, for all [L_T]:[Co_T] ratios studied, SDD shows that CoL was the predominant species (as examples, SDD for [L_T]:[Co_T] ratios 2.0 and 4.6 are shown in Figure 10-5), which was previously suggested by the agreement of the stability constants values refined from all [L_T]:[Co_T] ratios ($\log \beta_{\text{CoL}} = 2.2 \pm 0.1$) (Tables 4 and 5), unless [L_T]:[Co_T] ratio 1.7 recorded by UV-Vis. Additionally, SDD drawn for the higher [L_T]:[Co_T] ratios (here exemplified for [L_T]:[Co_T] ratio 4.6 (Figure 10-5B)) predict, prior to precipitation, the formation of an appreciable amount of CoL₂ (the maximum amount varied between 5 and 41% for all [L_T]:[Co_T] ratios) and of CoL(OH), as a minor species, but enough to allow the refinement of the stability constant value.

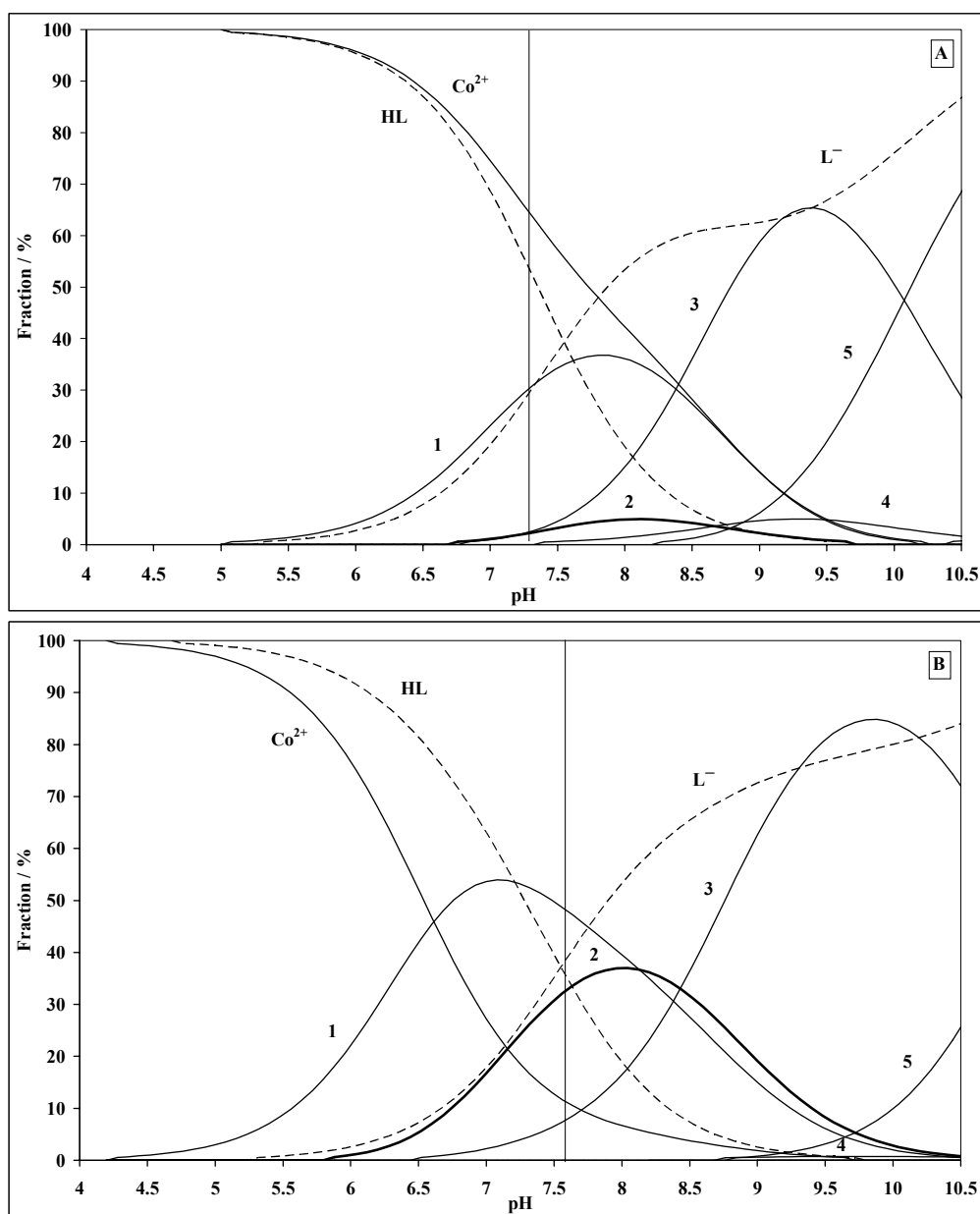


Figure 10-5 Species distribution diagrams plotted for the final model for Co-(TAPSO)_x-(OH)_y system: $\log \beta_{\text{CoL}} = 3.0$, $\log \beta_{\text{CoL}_2} = 4.8$ and $\log \beta_{\text{CoL(OH)}} = 7.6$. (A) $[\text{L}_\text{T}] : [\text{Co}_\text{T}] = 2.0$, $[\text{Co}^{2+}] = 4.6 \times 10^{-3} \text{ M}$ and (B) $[\text{L}_\text{T}] : [\text{Co}_\text{T}] = 4.6$, $[\text{Co}^{2+}] = 1.5 \times 10^{-2} \text{ M}$. The species included in the SDD are: 1 – CoL, 2 – CoL₂, 3- CoL(OH), 4 – Co(OH) and 5 – Co(OH)₂. Values for $\log \beta_{\text{Co}_x(\text{OH})_y}$ were from Table 10-1. The vertical line represents the pH at which precipitation of Co(OH)₂ was predicted.

10.3.2 MODELLING OF M^{2+} -(TAPS)_x-(OH)_y AND M^{2+} -(AMPSO)_x-(OH)_y SYSTEMS

In order to define Ni-(TAPS)_x-(OH)_y and Ni-(AMPSO)_x-(OH)_y systems, several $[\text{L}_\text{T}]:[\text{Ni}_\text{T}]$ ratios were performed by GEP. For Ni-(TAPS)_x-(OH)_y system, $[\text{L}_\text{T}]:[\text{Ni}_\text{T}] = 6.2$ and 15.0, with $[\text{Ni}^{2+}] = 5.0$

$\times 10^{-3}$ M, were done. For Ni-(AMPSO)_x-(OH)_y system, $[L_T]:[Ni_T] = 6.0$ and 8.5 with $[Ni^{2+}]$ equal to 8.3×10^{-3} M and 6.0×10^{-3} M, respectively, were tested. Note that so high $[L_T]:[M_T]$ ratios are not usually used in GEP experiments and the analysis of results should be carefully done. For both systems, the collected points used for characterizing the systems were only those for which $[L] \left/ \left[\sum_{x,y=0}^2 NiL_x(OH)_y \right] \right.$, where $x = 1$ or 2 and $y = 0, 1$ or 2 were not higher than 6 , otherwise all the titration goes towards the deprotonation of the ligand and not to neutralise protons liberated from the metal complexation (for example: $M + HL = ML + H$).

For both systems, an unequivocal interaction between the metal and the buffer was observed, as it is demonstrated by the analysis of Z_M function (Figure 10-2C). For Ni-(AMPSO)_x-(OH)_y system, the experimental Z_M function, generated for $[L_T]:[Ni_T] = 8.5$, increased up to 0.5 before precipitation of $Ni(OH)_2$ was detected around pH 8.0 . This pH corresponds just to the beginning of the buffering pH range (8.05 - 10.05). For Ni-(TAPS)_x-(OH)_y system, the Z_M function, generated for $[L_T]:[Ni_T] = 6.2$, grew linearly up to 1 , indicating the formation of NiL , and then a fast increase, typical of $NiL_x(OH)_y$ species, was observed. However, it was only possible to collect data up to pH 7.8 due to the early precipitation of $Ni(OH)_2$. For $[L_T]:[Ni_T] = 15$, precipitation occurred around pH 8.2 , which corresponds to half of the buffering pH-range region ($7.35 - 9.35$).

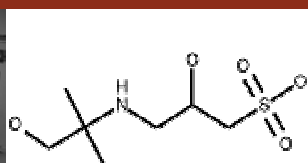
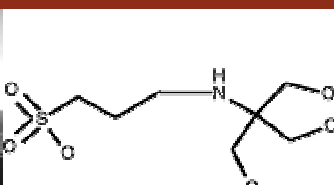
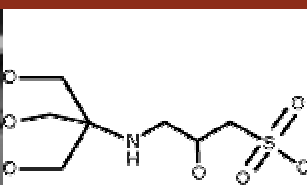
Even though a weaker complexation between TAPS and AMPSO with cobalt(II) was expected, as it occurred between TAPSO and cobalt(II), an attempt to characterize these systems was done. For this purpose, GEP titrations using $[L_T]:[Co_T] = 10.0$ ($[Co^{2+}] = 5.2 \times 10^{-3}$ M) and 17.0 ($[Co^{2+}] = 5.3 \times 10^{-3}$ M) were run for TAPS and AMPSO, respectively. Experimental points could only be collected up to pH 8.0 and 7.8 , for TAPS and AMPSO, respectively, due to precipitation of $Co(OH)_2$.

For all systems, no other experimental conditions were possible to be tested by GEP in order to define completely the $M-(L)_x-(OH)_y$ systems in the pH range where the buffers are used. As far as we know, there is no available techniques, which allow us to cover higher $[L_T]:[M_T]$ ratios with these type of ligands. It is important to note that these buffers are very simple ligands with no double bonds. So, UV-Vis and fluorescence, which are the only techniques that use low concentration of ligands (between 10^{-7} and 10^{-4} M), cannot be applied for these systems because they do not absorb. On the other hand, polarography that uses lower metal concentrations cannot be applied for these two metals because these metal systems are not electrochemically reversible. However, even though it was not possible a proper characterization of the $Ni^{2+}-(L)_x-(OH)_y$ and $Co^{2+}-(L)_x-(OH)_y$ systems, these results evidenced that TAPS and AMPSO complex nickel and cobalt. So these buffers should not be used for nickel or cobalt studies.

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PART VI GENERAL DISCUSSION, CONCLUSIONS AND FUTURE WORK

11 GENERAL DISCUSSION, CONCLUSIONS AND FUTURE WORK

11.1 GENERAL DISCUSSION

Adsorption studies of the ligands TAPSO, AMPSO and TAPS at the mercury electrode pointed out a slight adsorption occurrence, for all three buffers, in the potential range between 0.2 and -0.7 V, being the maximum adsorption recorded in the region of zero charge potential, where lead reduction occurs. This effect was more pronounced for TAPS followed by TAPSO and AMPSO. In a general way, the largest adsorption occurred for $\text{pH} = \text{p}K_a$ or $\text{pH} = \text{p}K_a + 1$, which corresponds to the pH range where the deprotonated form of the ligand predominates. This fact suggested that, at this potential, the adsorption of the ligands was mainly due to the uncharged part of the ligands.

In this thesis, the interaction between three pH buffers, TAPSO, TAPS and AMPSO ($\text{p}K_a$ values between 7.47 and 9.05), and six divalent metals ions (copper, lead, cadmium, zinc, cobalt and nickel) were studied. The results obtained clearly indicated complexation, with different strength, for all $\text{M}-(\text{buffer})_x-(\text{OH})_y$ systems. The final models of thirteen $\text{M}-(\text{buffer})_x-(\text{OH})_y$ systems, were fully defined whereas the complete characterization, in the buffer pH range, of five of these $\text{M}-(\text{buffer})_x-(\text{OH})_y$ (between cobalt or nickel and TAPS and cobalt or lead or nickel and AMPSO) systems was not possible due to the precipitation of metal hydroxide species. The final models, as well as the overall stability constants, are summarized in Table 11-1.

The comparison between the different metals studied showed that the stability constant values follow the order: $\text{Cu}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$, a modified Irving-Williams series, where Pb^{2+} and Cd^{2+} ions were included. The comparison between ligands evidences that an increase in bound sites of the ligand, namely -OH groups (all of the ligands have an amine group as favourite coordinate site) increases $\log \beta_{\text{ML}}$, as it was evidenced for TAPSO with copper, lead and zinc.

Table 11-1 shows that, for each metal ion and independently of the buffer under study, some species were systematically detected (see highlighted species in Table 11-1).

Table 11-1 Final models and stability constants values (as $\log \beta$) for all $M-(L)_x-(OH)_y$ systems studied.

Ligand (L)		TAPSO	TAPS	AMPSO
<i>pKa</i>		7.55	8.342	9.05
Cu²⁺	CuL	4.41	4.2	4.62
	CuL(OH)	11.43	-	11.7
	CuL(OH) ₂	17.55	-	-
	CuL₂	8.08	7.8	9.5
	CuL₂(OH)	14.3	13.9	15.6
	CuL₂(OH)₂	20.3	18.94	21.2
	CuL ₃	-	-	13.4
Pb²⁺				C, *
	PbL	3.7	3.29	
	PbL ₂	6.6	-	
	PbL₂(OH)	12.9	12.1	
	PbL₂(OH)₂	17.8	17.0	
Ni²⁺			C, *	C, *
	NiL	2.99		
	NiL ₂	4.8		
Zn²⁺	ZnL	2.5	2.3	2.5
	ZnL(OH)	7.2	-	-
	ZnL(OH)₂	13.2	13.0	12.9
Co²⁺			C, *	C, *
	CoL	2.2		
	CoL(OH)	7.6		
	CoL ₂	3.6		
Cd²⁺	CdL	2.2	2.40	2.1
	CdL(OH)	-	-	6.2
	CdL ₂	4.2	-	-
	CdL ₂ (OH)	8.6	-	-

C- occurrence of complexation; * - it was not possible to define this systems

For $\text{Cu}-(\text{buffer})_x-(\text{OH})_y$ systems, different models were identified for each system, where species like CuL , $\text{CuL}(\text{OH})_y$, CuL_2 , $\text{CuL}_2(\text{OH})_y$ and CuL_3 were part of the final models; final models and the strength of the overall stability constants varied according to the structure and the pK_a value of the buffer. An evaluation based on $\log \beta_{\text{CuL}}$ versus pK_a suggested that TAPS and AMPSO coordinated via amino and hydroxymethyl groups forming a five-membered chelate ring. TAPSO is most likely a tridentate ligand, coordinating by the amino group and by two hydroxyl groups, forming two five-membered chelate rings. The comparative analysis of the models for TAPSO and AMPSO pointed out, as a major difference, that TAPSO was not able to form CuL_3 . This fact is probably related with the crowded atoms arrangement on the nitrogen of TAPSO, which does not allow this ligand to form more than CuL_2 .

For $\text{Pb}-(\text{buffer})_x-(\text{OH})_y$ systems, the final models did not differ considerably; PbL , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$ species were identified for both $(\text{Pb}-(\text{TAPSO})_x-(\text{OH})_y)$ and $\text{Pb}-(\text{TAPS})_x-(\text{OH})_y$ systems characterized. A comparative analysis of the strength of $\log \beta_{\text{PbL}}$, for both systems, suggests that the TAPSO's hydroxyl group near the sulfonic group is involved in the coordination, forming a five-membered chelate ring and increasing the stability of the PbL complex.

For $\text{Zn}-(\text{buffer})_x-(\text{OH})_y$ systems, ZnL and $\text{ZnL}(\text{OH})_2$ are the species that constitute the final models for all the three systems studied.

The final models for $\text{Cd}-(\text{buffer})_x-(\text{OH})_y$ systems varied according to the buffer's pK_a . CdL was identified for all three systems. Due to the weakness of the complexes and the high basicity of TAPS and AMPSO, whose deprotonation occurs in the region of cadmium hydrolysis, CdL_2 was only detected for $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ system. $\text{CdL}_x(\text{OH})$ species were identified for $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ and $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ systems. For $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ and $\text{Co}-(\text{TAPSO})_x-(\text{OH})_y$ systems, the proposed final models were NiL and NiL_2 and CoL , CoL_2 , $\text{CoL}(\text{OH})$, respectively. The formation of $\text{CoL}(\text{OH})$ species was characterized by a major structural arrangement (Co^{2+} binding site changed from an octahedral towards a tetrahedral geometry).

Metal ion speciation studies of natural waters are performed at constant pH. For this purpose, a pH buffer is usually added in a concentration that can range between 0.005 and 0.1M. The pH values of natural waters, reported in the literature [1, 2], varies according to the type of water. For freshwaters, the pH varies between 5.7 and 9.9 and for sea and estuarine waters it ranges from 7.3 to 8.4. This means that all the buffers studied in this thesis, TAPSO, TAPS and AMPSO, can be potential pH buffers to be used in metal speciation studies when researchers search a "proper" buffer for their studies.

Filella and Town performed a meticulous survey of the binding parameters (effective ligand concentration and conditional stability constants), reported in the literature, using the same experimental conditions and the same interpretative mode of trace metals (Cu^{2+} , Zn^{2+} , Pb^{2+} and Cd^{2+}) in seawater, estuarine and freshwater samples [1, 2].

The conditional stability constants of the organic complexes of copper(II) reported range between (as $\log K$) 4 and 16 (with the most frequent values ranging between 8 and 15), with a variation of the ligand concentration from 1 to 10000nM [1, 2]. For zinc, the conditional stability constants values range from 7 to 11.5 (being the most common values between 7 and 9.5 [2]), with ligand concentrations between 1 and 3162nM [1, 2]. A variation in the ligand concentration from 0.03 to 1000nM and a range in conditional stability constants of the lead organic complexes between 6 and 13 are observed [1, 2], whose most frequent values occur between 9.5 and 12.5 [2]. The reported cadmium binding parameters show a variation of the conditional stability constants (as $\log K$) between 7 and 11 (with a clear vertical section between 10–11) and a variation of the ligand concentration between 0.03 and 10nM [1, 2]. For cobalt and nickel the information regarding binding constants in natural systems is scarce. The binding stability constants reported in the literature are high [3, 4], but usually, these metal ions are only partially bounded to the organic ligands [3]. For nickel, conditional stability constants as high as 12.1 – 14.9 ($\log K$ values) and organic ligand concentrations between 13 and 100nM were reported in freshwaters [5]. A recent cobalt speciation study, in seawater, presented values for complexation to natural organic ligands with conditional stability constants ranging between 15.6 and 16.1 and ligand concentrations between 22 and 60pM [6].

Table 11-1 evidences that the magnitude of the stability constants (as $\log \beta$) determined for the various $\text{M}-(\text{buffer})_x-(\text{OH})_y$ systems ranges between 2.1 (CdL), for $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ system, and 21.3 ($\text{CuL}_2(\text{OH})_2$), for $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system.

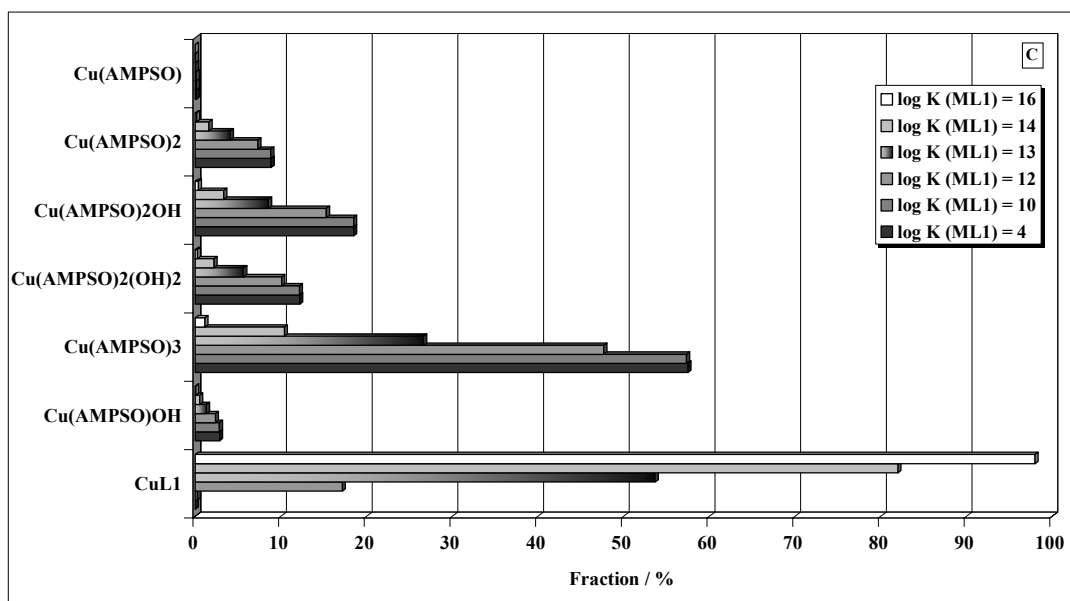
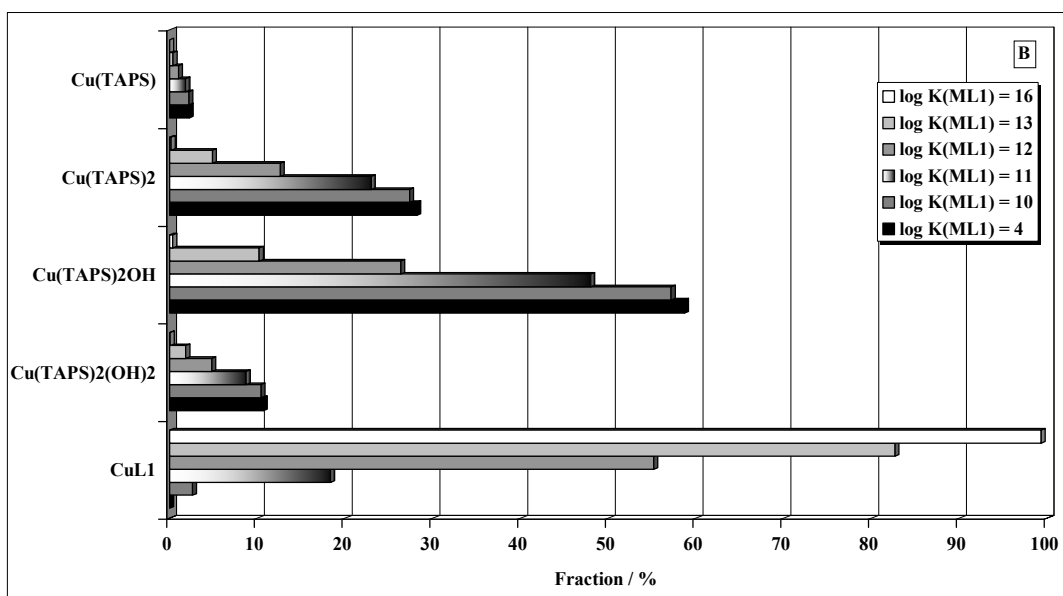
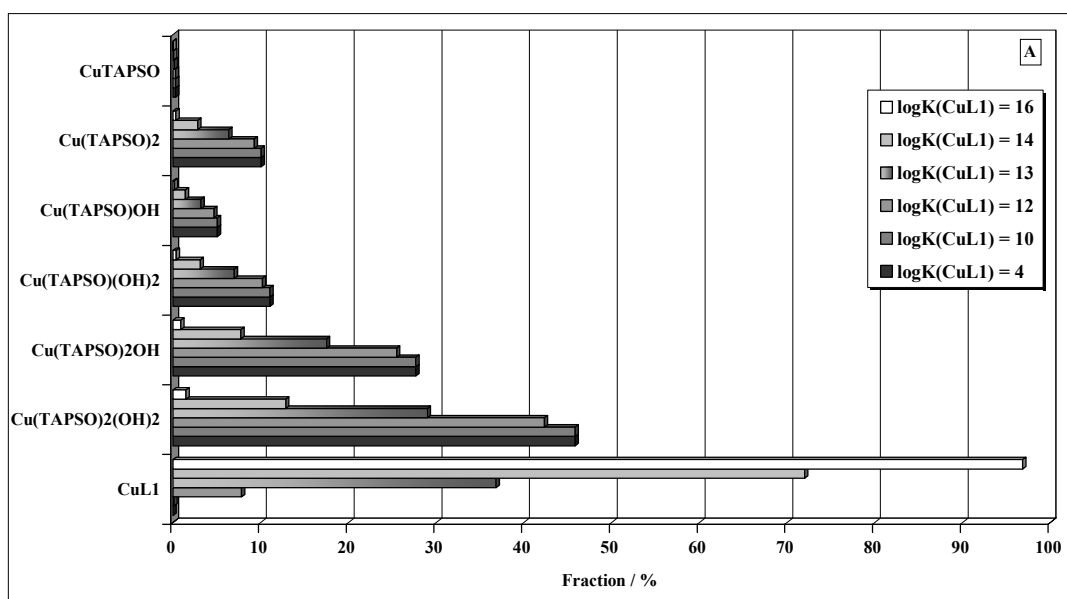
When we compare the magnitude of these stability constants values with the conditional stability constants reported in the literature for trace metals (Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+}) determined in natural water samples [1, 2], it becomes clearly that the use of these buffers can modify the scenario of metal ion speciation. For cobalt and nickel, due to the large conditional stability constants described in the literature, this effect will probably only affect the inorganic metal ion speciation.

In order to evaluate the possible interference, by a competitive ligand as is the case of the pH buffer, chemical equilibrium simulations were performed using the SolEq software (Solution Equilibria Principles and Applications). For this purpose, two ligands were considered: one representing the natural organic ligands (here represented by L_1), whose

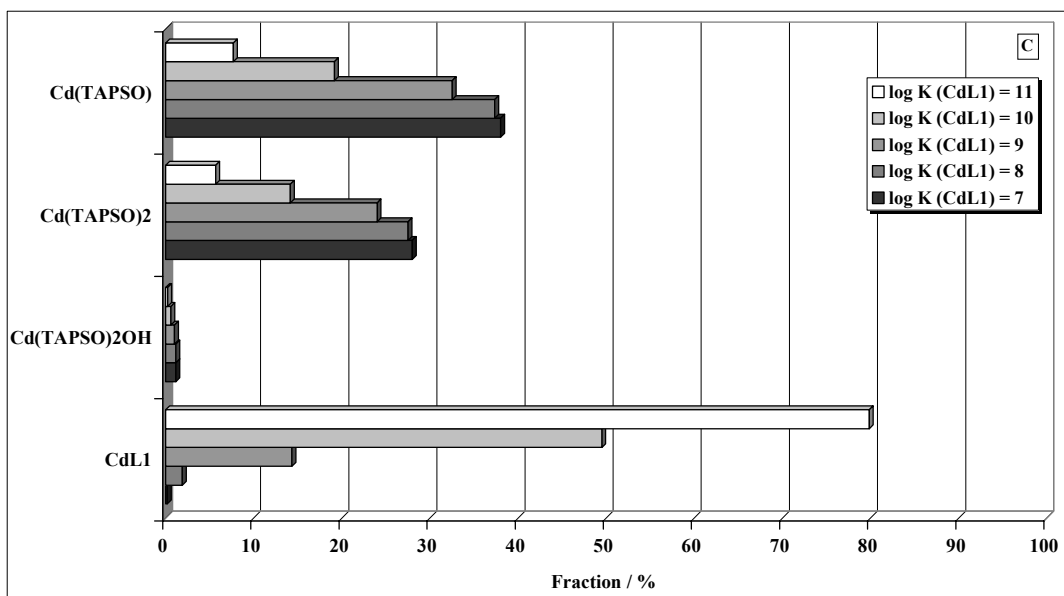
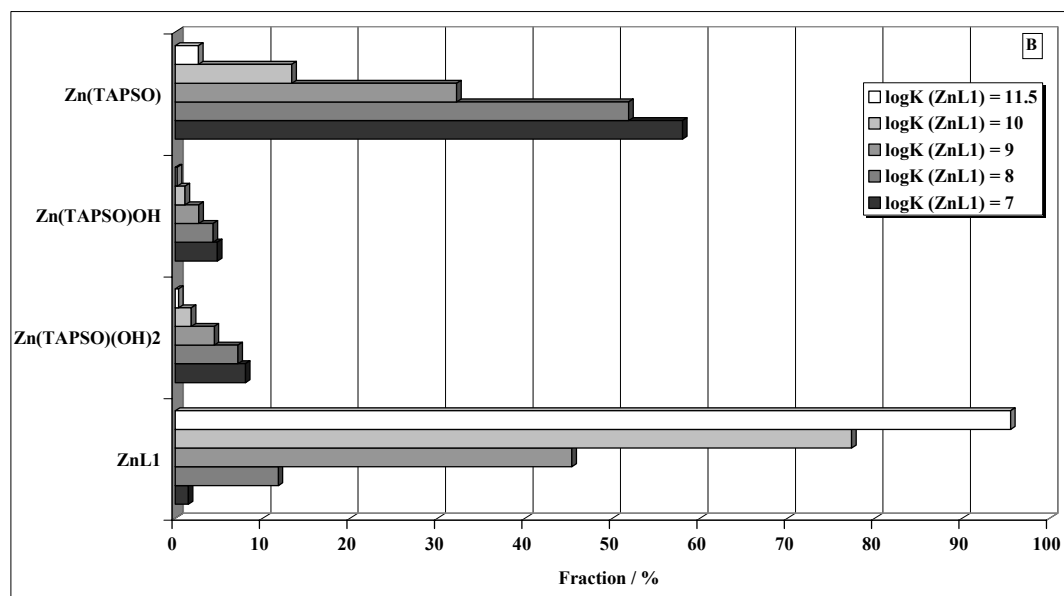
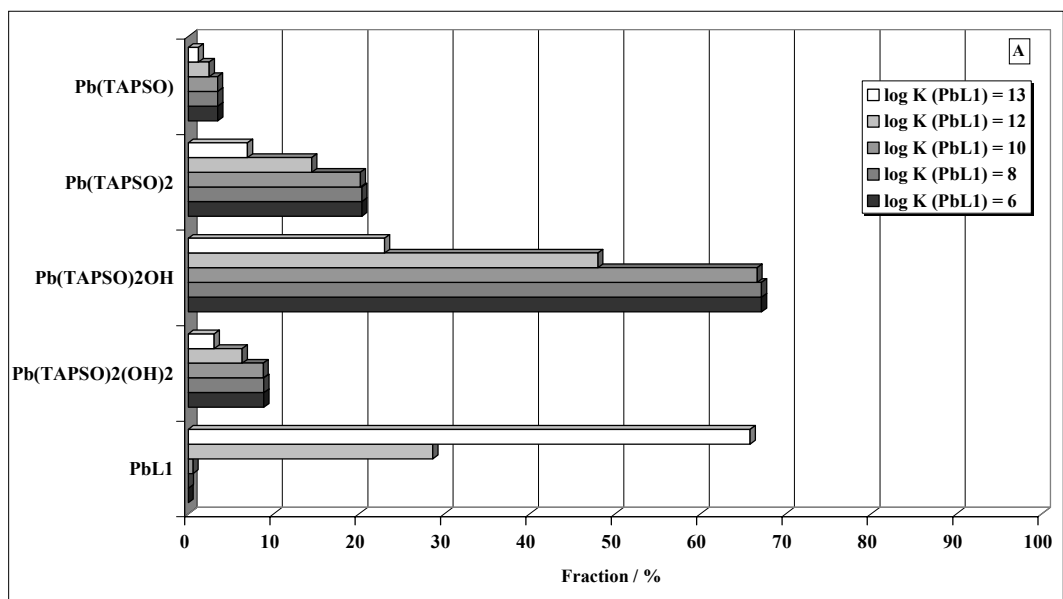
conditional stability constants were varied according to the values reported in the literature [1, 2], and the pH buffers studied in this work. Besides, hydroxide, no other ligands were considered. Eleven $M-(\text{buffer})_x-(\text{OH})_y$ systems (divalent metal ions simulated were: Cu, Pb, Zn and Cd) were simulated using 0.01M of pH buffer [TAPSO, TAPS or AMPSO (only for Cu, Zn and Cd)] at pH 8.0. For each $M-(\text{buffer})_x-(\text{OH})_y$ system, equivalent total concentrations of natural ligands ($[L_1]$) and metal ion ($[M^{n+}]$) were simulated: (i) $[L_1]$ or $[Cu^{2+}] = 6.0\text{nM}$; (ii) $[L_1]$ or $[Pb^{2+}] = 0.6\text{ nM}$; (iii) $[L_1]$ or $[Zn^{2+}] = 6.0\text{nM}$ and $[L_1]$ or $[Cd^{2+}] = 0.6\text{ nM}$. Examples of the competitive effect between the three pH buffers and the natural organic ligands in the presence of copper is shown in Figure 11-1. Some additional examples were also included for lead, cadmium and zinc in the presence of TAPSO (Figure 11-2); similar results were obtained with the other five $M-(\text{Buffer})_x-(\text{OH})_y$ systems involving TAPS and AMPSO (data not shown).

A global analysis of the simulation studies evidenced that all pH buffers would influence the speciation of all four metal ions. A more marked effect occurs for lead, followed by cadmium and then by copper and zinc in a similar extent. A comparative analysis between the three buffers shows that TAPSO influences more copper speciation than TAPS and AMPSO, being the influence of these two buffers in a similar extent (Figure 11-1 and Figure 11-2). For all three buffers and up to $\log K(\text{CuL}_1) = 12$, about 80% of copper is complexed with the pH buffer; this ligand competition effect is mainly due to $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$, for TAPSO, CuL_2 and $\text{CuL}_2(\text{OH})$, for TAPS, and CuL_3 , for AMPSO. For $\log K(\text{CuL}_1) \geq 14$, more than 70% corresponds to CuL_1 species for all three systems studied. For the other metals (cadmium, lead and zinc), TAPSO influences more the metal ion speciation than TAPS or AMPSO (data not shown). For both pH buffers (TAPSO and TAPS) and up to $\log K(\text{PbL}_1) = 10$, pH buffers bind lead stronger ($> 80\%$) than the natural organic ligands; this complexation is mainly due to the formation of $\text{PbL}_2(\text{OH})$ species. For zinc and cadmium, an amount of metal-buffer species higher than 70% was predicted, for all three buffers (for TAPS and AMPSO, data not shown), when $\log K(\text{ML}_1) \leq 8$ and 9, respectively. This pH buffer competition is mainly due to ML formation unless for Cd-TAPSO, where a large amount of CdL_2 also occurs (Figure 11-2).

Considering the frequency of $\log K(\text{ML}_1)$ reported in the literature for natural ligands [2], the simulations presented above clearly demonstrated that all three buffers can interfere to a significative extent in metal speciation studies performed in natural waters.



← **Figure 11-1** Fraction of each species in a system containing 0.01 M buffer at pH 8.0 in the presence of a equivalent molar concentration, 6.0 nM, of natural organic ligands (L1) and copper: (A) TAPSO; (B) TAPS; (C) AMPSO.



← **Figure 11-2** Fraction of each species in a system containing 0.01 M TAPSO at pH 8.0 in the presence of equivalent molar concentration of natural organic ligands (L1) and the following metal ions: (A) [L1] or [Pb²⁺] = 0.6 nM; (B) [L1] or [Zn²⁺] = 6.0 nM TAPS; (C) [L1] or [Cd²⁺] = 0.6 nM.

11.2 CONCLUSIONS

Complexation properties of eighteen $M-(\text{buffer})_x-(\text{OH})_y$ systems involving six divalent metal ions (cadmium, cobalt, copper, lead, nickel and zinc) and three pH buffers (TAPSO, TAPS and AMPSO) were characterized. Complexation was detected for all eighteen $M-(\text{Buffer})_x-(\text{OH})_y$ systems studied but fully definition of the final models was only possible to be performed for thirteen systems. For systems involving cobalt or nickel and TAPS and cobalt or lead or nickel and AMPSO, the definition of the systems, in the buffering pH-range, was not possible due to the precipitation of the metal hydroxide species.

The final models and the values of the overall stability constants varied according to the structure of the buffer and the metal ion. The order of strength of the bound metal–buffer was: $\text{Cu}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$. The magnitude of the stability constant of ML species (as $\log \beta_{\text{ML}}$) ranged between 4.62, for $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system and 2.1, for $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ system.

For $\text{Cu}-(\text{buffer})_x-(\text{OH})_y$ systems, CuL , CuL_2 , $\text{CuL}_2(\text{OH})$ and $\text{CuL}_2(\text{OH})_2$ were identified for all three systems studied. For both $\text{Pb}-(\text{buffer})_x-(\text{OH})_y$ systems characterized, PbL , $\text{PbL}_2(\text{OH})$ and $\text{PbL}_2(\text{OH})_2$ were always detected, whereas for $\text{Zn}-(\text{buffer})_x-(\text{OH})_y$ and $\text{Cd}-(\text{buffer})_x-(\text{OH})_y$ systems, only ZnL and $\text{ZnL}(\text{OH})_2$ and CdL were identified for all three $M-(\text{buffer})_x-(\text{OH})_y$ systems. The presence of other species were detected in a randomly way; ML_2 was detected for $\text{Ni}-(\text{TAPSO})_x-(\text{OH})_y$ and $\text{Cd}-(\text{TAPSO})_x-(\text{OH})_y$ systems and CuL_3 was identified for $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$ system. $\text{ML}(\text{OH})_x$ species were identified for $\text{Cu}-(\text{TAPSO})_x-(\text{OH})_y$, $\text{Cu}-(\text{AMPSO})_x-(\text{OH})_y$, $\text{Zn}-(\text{TAPSO})_x-(\text{OH})_y$ and $\text{Cd}-(\text{AMPSO})_x-(\text{OH})_y$ systems.

Chemical species simulation studies, involving the metal stability constants determined for the three buffers studied in this thesis and the conditional stability constants reported in the literature for natural water samples (Cd^{2+} , Cu^{2+} , Pb^{2+} and Zn^{2+}) [1, 2], demonstrated that all buffers would compete to a significative extent in metal speciation studies performed in natural waters, at $\text{pH} = 8.0$. A more marked effect was predicted for lead, followed by cadmium and then by copper and zinc in a similar extent. A comparative analysis between the three buffers shows that TAPSO compete more for metal natural binding sites at $\text{pH} = 8.0$ than TAPS or AMPSO.

11.3 SUGGESTIONS FOR FUTURE WORK

With the conclusion of the present work, there are other commercial pH buffers, which were not yet studied. As a future work, complexation studies between CAPS, CAPSO, CHES and DIPSO and the various metal ions are proposed.

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"Genius is one per cent inspiration, ninety-nine per cent perspiration."

Thomas Alva Edison (1847-1931); US physicist and inventor.